University of Alberta

Interactions among *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), brassicaceous and non-brassicaceous host plants, and its larval parasitoids

by

©

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Abstract

Interactions among the insect herbivore *Plutella xylostella* (L.), various plant species, and its parasitoids *Diadegma insulare* (Cresson) and *Microplitis plutellae* (Muesebeck) were studied in experiments designed to investigate bitrophic and tritrophic responses to soil fertility and host plant genotype. Different fertilizer applications significantly affected the nutrient contents of *Brassica napus* (L.) foliage, and this in turn affected performance of *P. xylostella* and *D. insulare*. Female *P. xylostella* discriminated among host plant fertility levels for oviposition, and selected plants on which pre-imaginal survival and development of their offspring was maximal, and on which new generation adults had highest longevity when food was limited. Host plant nutrient regime on which *P. xylostella* host larvae were reared also affected various developmental parameters of *D. insulare*. Regardless of soil fertility rate, plants responded to herbivory by increasing root mass relative to their non-infested counterparts. Bottom-up effects of host plant resistance on both *P. xylostella* and *D. insulare* were investigated through examination of several life history parameters when insects were reared on eight genetically different Brassicaceae, some of which were conventional hosts and others were herbicide-tolerant genotypes. *Plutella xylostella* is oligophagous, with a natural host plant range restricted to the Brassicaceae; the present study investigated the ecological costs and benefits that can arise when a herbivore species maintains the genetic plasticity within its population so it can occasionally shift from its normal hosts to exploit non-host species. Seasonal distribution patterns of *P. xylostella*, *D. insulare* and *M. plutellae* were investigated in commercial fields of canola in southern Alberta. This study used geographical information systems to determine the

relationship between host plant quality, as assessed by tissue nutrient content, and field distributions of the herbivore and its parasitoids. Sampling *P. xylostella, D. insulare* and *M. plutellae* from points arranged in two grid patterns, together with the mapping and analysis of their spatial distributions over time generated a detailed picture of the pattern of crop colonization by the herbivore and its parasitoids. The studies have implications for improved management strategies for *P. xylostella* when the complex interactions among the different trophic levels are considered.

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Chapter 1:

General Introduction[‡]

Part-I: Diamondback Moth-Host Plant Interactions

1.1 Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a well known and destructive insect pest of brassicaceous crops worldwide. It globally requires over US $1.0 billion in estimated annual management costs (Javier, 1992; Talekar and Shelton, 1993) in addition to the crop losses it causes. This crucifer specialist may have its origin in Europe (Hardy, 1938), but on the basis of the presence of its host plants and biocontrol agents (175 species of plants and 14 species of parasitoids), Kfir (1998) speculated that it originated in South Africa. Using similar arguments, Liu et al. (2000) proposed that *P. xylostella* originated in China. This pest is now present wherever its host plants exist and is considered to be the most broadly distributed of all Lepidoptera (Shelton, 2004).

In the southeastern United States, *P. xylostella* constitutes more than 90% of the guild of defoliating lepidopterans of canola (Buntin, 1990; Ramachandran et al., 2000). In Texas, it can cause losses of ca. US $40 to 70 million for cabbage and US $400,000 for broccoli crops if not treated (Shelton, 2004). Without treatments, much of the ca. US $500 million broccoli crop in California and the ca. US $80 million cabbage crop in New York would be unmarketable (Shelton, 2004). In Australia, its control on cabbage and canola requires ca. $A12 million and ca. $A6 million, respectively (Shelton, 2004). Outbreaks of *P. xylostella* in Southeast Asia sometimes cause more than 90% crop loss (Verkerk and Wright, 1996). In Shanghai (China), when no insecticides were applied to control *P. xylostella* in 1992 and 1994, the losses of summer cabbage were 99 and 80%, respectively (Zhao et al., 1996). In India, it causes annual losses of ca. US $16 million (Mohan and Gujar, 2003a). In certain parts of the world including Nicaragua and Pakistan, *P. xylostella* infestations sometimes compel growers to plough down their standing crops in spite of multiple insecticide applications (Abro et al., 1994; Pérez et al., 2000).

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Plutella xylostella is also a serious pest of Brassicaceae in Canada. It was likely introduced from Europe before 1854 (Anonymous, 1996) and now occurs annually throughout Canada wherever brassicaceous crops are grown and can cause substantial crop losses during outbreak years (Harcourt, 1957; Dosdall et al., 2004b). In 1985, ca. 467,860 ha were treated with insecticides in Alberta and Saskatchewan to control an outbreak at an estimated cost of CN $11.9 million (Madder and Stemeroff, 1988). In 1995, ca. 1.25 million ha were treated to control another outbreak at an estimated cost of CN $50 million (WCCP, 1995). In 2001, ca. 1.8 million ha were sprayed with insecticides in western Canada for control of crucivores primarily *P. xylostella* (WCCP, 2001). The source of *P. xylostella* in western Canada is attributed to influxes from southerly regions (Smith and Sears, 1982; Dosdall et al., 2004b). Similar migrations have been reported in the U. K. (Hardy, 1938; Chapman et al., 2002), New Zealand, South Africa, and southern parts of Chile and Argentina (Talekar and Shelton, 1993), Japan (Honda, 1992) and Australia (Goodwin and Danthanarayana, 1984). Moths are highly migratory and have been recorded to travel a distance of about 1500 km at 400 to 500 km per night (Chapman et al., 2002).

Plutella xylostella is multivoltine with 4 to 20 generations per year in temperate and tropical regions, respectively (Harcourt, 1986; Ferreira and Torres, 2003; Vickers et al., 2004). Each female can lay over 200 eggs mainly on the upper leaf surface (Talekar et al., 1994; Justus et al., 2000). Eggs hatch after 4 to 8 days and first-instar larvae usually mine the spongy mesophyll tissues (Harcourt, 1957). Second-, third- and fourth-instar larvae are surface feeders and they consume leaves, buds, flowers, siliques, the green outer layer of stems and also developing seeds within older siliques (Anonymous, 1996). The average duration of larval instars under field conditions in eastern Ontario (Canada) was 4.0, 3.6, 3.4, and 4.2 days for the first through fourth instars, respectively, and pupation required 7.8 to 9.8 days (Harcourt, 1957). The observation that as many as 20 generations per year can occur in the tropics indicates that developmental time from egg to adult is much more rapid in those regions. Successful development can occur at constant temperatures from 8°C to 32°C and under alternating regimes including temperature as low as 4°C or as high as 38°C (Liu et al., 2002). There is also evidence for its overwintering in temperate areas of the world including the U.S.A. (Marsh, 1917), the U.K. (Hardy, 1938) and Canada (Dosdall, 1994).

Integrated pest management programs for *P. xylostella* comprise mainly chemical, biological and cultural control tactics. In certain brassicaceous vegetable growing areas (e.g. India) ca. 50 to 60 applications of insecticides are made every year (Zhao et al., 2002; Samsudin et al., 2004). Under such high selection pressure, pests often develop high levels of insecticide resistance and *P. xylostella* is one of the "leaders" among insect pests that are very difficult to control (Mota-Sanchez et al., 2002; Sarfraz and Keddie, 2005). It was the first crop insect reported resistant to DDT in 1953 in Indonesia (Johnson, 1953) and now in many crucifer-producing regions it has shown significant resistance to almost every synthetic insecticide applied in the field including relatively new chemistries such as avermectins, macrocyclic lactones, neo-nicotinoids, oxadiazines, pyrazoles, insect growth regulators and nereistoxin analogue insecticides (Yu and Nguyen, 1992; Shelton and Wyman, 1992; Mohan and Gujar, 2003a; Ninsin, 2004; Sayyed et al., 2004; Sarfraz and Keddie, 2005, Sarfraz et al., 2005a). Due to insecticide resistance and eventual control failure of *P. xylostella*, economical production of crucifers has become almost impossible in certain areas of the world (e.g. parts of Southeast Asia, Central America, the Caribbean, and the southeastern United States) (Talekar and Shelton, 1993). This has prompted evaluation of alternative pest management strategies, comprising mainly cultural and biological control.

In this chapter, I synthesize published information on *P. xylostella* and its host plants and focus on aspects of this relationship relevant for its control, especially in relation to host plant resistance, genetic modification of crop plants and trap cropping. The biochemical and morphological bases of antixenotic and antiobiotic resistance, and their contribution to *P. xylostella* management, are discussed. I summarize the potential efficacies of conventional and dead-end trap cropping, and the potential usefulness of genetically modified crop plants for the sustainable management of pest populations of *P. xylostella*.

1.2 *Plutella xylostella*: A Crucifer Specialist

1.2.1 Oviposition Specialization

Plutella xylostella is likely attracted to its brassicaceous host plants by chemical (olfactory/gustatory) and/or physical (tactile/visual) stimuli (Justus and Mitchell, 1996; Badenes-Perez et al., 2004; Shelton, 2004; Bukovinszky et al., 2005). Behaviors associated with host plant acceptance and oviposition comprise antennal rotation, antennation and ovipositor sweep (see Justus and Mitchell, 1996). Once their host plant is found, the moths exhibit a strong arrestment response by staying or hopping to neighboring plants (Bukovinszky et al., 2005). Cues from the host plant stimulate the onset of reproductive activities of *P. xylostella*. The presence of host plants accelerated calling behavior and females began calling at a younger age, began calling earlier in the scotophase and spent more time calling (Pittendrigh and Pivnick, 1993). Host cues accelerated egg maturation, increased the incidence of mating and shortened the time between adult emergence and the onset of oviposition (Hillyer and Thorsteinson, 1971; Pivnick et al., 1990). However, the presence of non-host plants such as wheat, broad beans and peas did not significantly affect the length of the prereproductive period (Hillyer and Thorsteinson, 1969).

1.2.2 Herbivory Specialization

The feeding activity of generalist insects is often stimulated by nutrients such as sugars, amino acids and other primary metabolites that occur in nearly all plants. In contrast, specialist phytophagous insects such as *P. xylostella* usually require additional, more specific chemical cues for feeding (Renwick and Lopez, 1999), and their host range is limited to a particular group of plants (see Section 1.3.1.1). Food plant range is determined not only on the basis of adequate feeding stimulants but also the frequency of certain feeding deterrents and/or toxic factors. If certain non-host plant species are devoid of any strong deterrent or toxicant compounds but are naturally deficient in decisive stimulants, their acceptability to *P. xylostella* can be recovered after treatment with basic stimulant(s) (Gupta and Thorsteinson, 1960a; Renwick and Radke, 1990; van Loon et al., 2002). In general, *P. xylostella* larvae are reliant on their mothers for host selection and sometimes may feed on 'neutral' non-host plants (e.g. sugar snap peas, *Pisum sativum* L. (Fabaceae)) at the time of hatching, and may even complete their development on these plants (Löhr, 2001; Löhr and Gathu, 2002) (Table 1-III). If the plant is not suitable, neonate larvae may not feed on it at all (Gupta and Thorsteinson, 1960a).

1.3 Host Plant Resistance/Susceptibility

Certain plant characteristics including biochemical or morphological factors, or a combination of both may promote resistance (antibiosis, antixenosis, or both antibiosis and antixenosis) to *P. xylostella*. Plants responsible for antibiosis may cause reduced insect size or weight, or have an indirect effect by increasing the exposure of the insect to its natural enemies as a result of prolonged developmental time. Plants that exhibit antixenotic resistance would have reduced initial infestations or a higher emigration rate of the pest than their susceptible counterparts (Dent, 2000).

1.3.1 Biochemical Bases of Resistance/Susceptibility

The biochemical bases of host plant resistance to *P. xylostella* can be divided into two broad categories, those influencing behavioral responses and those influencing the physiological responses. Behavior-modifying chemicals may be attractants, arrestants, deterrents, stimulants and/or repellents, while plant chemicals affecting the physiological processes of insects may be nutrients, physiological inhibitors and/or toxicants. The range of responses elicited by chemicals and their effects on insects are quite diverse and complex. Several types of plant chemicals have been investigated to determine their roles in promoting resistance to *P. xylostella*.

1.3.1.1 Glucosinolates

Brassicaceae (= Cruciferae) comprise a diverse group of 350 genera and over 3,500 species of dicotyledonous herbs (Warwick et al., 2003). The natural host range of *P. xylostella* includes cultivated and wild crucifers (Tables 1-I and 1-II) that are characterized by secondary plant compounds, the glucosinolates. Glucosinolates may be toxic to generalist insects (Blau et al., 1978; Bodnaryk, 1997; Li et al., 2000; Ratzka et al., 2002), but *P. xylostella* is known to rely on some of them for host location, oviposition and feeding stimulation (Thorsteinson, 1953; Gupta and Thorsteinson, 1960b; Reed et al., 1989; Justus and Mitchell, 1996; Ratzka et al., 2002; Marazzi et al., 2004; Shelton, 2004).

Glucosinolates are non-volatile nitrogen and sulfur-containing glycosides derived from amino acid precursors and known to occur in 16 families of dicotyledonous angiosperms including Brassicaceae (Charron and Sams, 2004); more than 100 different glucosinolates have been identified (Rask et al., 2000). Their biosynthesis occurs in three independent stages viz., chain elongation of the amino acid, formation of the core structure (including a β-thioglucose moiety and a sulfonated oxime), and side chain modifications (Kroymann et al., 2003). Glucosinolates are divided into three main classes including (1) those with side chains which may contain aliphatic allylic or aliphatic non-allylic groups, (2) those possessing side chains comprising the indolyl group, and (3) those with aromatic side chains (Louda and Mole, 1991; Mithen, 1992; Rask et al., 2000; Bogdanov, 2004). Generally, progoitrin and gluconapoleiferin predominate in canola, *Brassica napus* L., sinigrin in Indian mustard, *Brassica juncea* (L.), garlic mustard, *Alliaria petiolata* (Bieb), and thalecress, *Arabidopsis thaliana* (L.) (Cvi-0 ecotype), glucobrassicin in cabbage, *Brassica oleracea* L., sinalbin and glucosinalbin in white mustard, *Sinapis alba* L., and glucobarbarin in yellow rocket, *Barbarea vulgaris* (L.) R. Br. (Renwick and Lopez, 1999; Ratzka et al., 2002; Marazzi et al., 2004, Bogdanov, 2004).

1.3.1.1.1 Oviposition and Feeding Stimulants

Certain glucosinolates including sinigrin and glucobrassicin and/or their metabolites that occur in Brassicaceae are stimulatory to *P. xylostella* for oviposition (Gupta and Thorsteinson, 1960b; Reed et al., 1989; Renwick and Radke, 1990; Talekar and Shelton, 1993; Shelton, 2004). Glucosinolates differ in their side chains even within one group and some of them, such as glucotropaeolin, apparently are not stimulatory to *P. xylostella* for oviposition (Renwick and Radke, 1990). Reed et al. (1989) reported that intact glucosinolates stimulated oviposition and their degradation with myrosinase or sulfatase largely eliminated the activity. *Plutella xylostella* exhibited significantly higher and consistent oviposition on sinigrin-treated leaves of Sieva bean plants compared with untreated control plants (Renwick and Radke, 1990). Aqueous extracts of two cultivated crucifers viz., *B. oleracea*, *B. juncea* and a wild crucifer, *Erysimum cheiranthoides* L., stimulated *P. xylostella* oviposition when applied to bean plants while extracts of *Tropaeolum majus* L. (Tropaeolaceae) yielded only a limited enhancement effect (Renwick and Radke, 1990).

The glucosides sinigrin, sinalbin and glucocheirolin act as specific feeding stimulants for *P. xylostella* and over 40 plant species containing one or more of these compounds serve as hosts (Talekar and Shelton, 1993; Shelton, 2004). Leaf discs of 12 non-cruciferous plants (including *Dahlia* sp., *Gynura* sp., *Chrysanthemum* sp., *Cucumis* sp., *Euphorbia poinsettiana* Buist., *E. splendens* Mill, *Abutilan* sp., *Maranta* sp., *Pepromia* sp., *Rhamnus* sp., *Clematis* sp. and *Rosa* sp.) treated with sinigrin were readily accepted by *P. xylostella* neonate larvae (Gupta and Thorsteinson, 1960a) suggesting that these plant species are deficient in essential feeding stimulants but may contain relatively weak feeding deterrents or none at all. Feeding activities of *P. xylostella* did not differ significantly with high glucosinolate concentrations in *B. juncea* and glucosinolates added to artificial diet were not toxic to its neonates (LC_{50} >>100μmol g^{-1}) (Li et al., 2000). Sinigrin concentrations in 18-day-old leaves of *B. juncea* cv. Cutlass were significantly higher than two low-glucosinolate genotypes and foliar concentrations of sinalbin were higher in *S. alba* cv. Gisilba than a selected line (Bodnaryk, 1997). *Plutella xylostella* feeding rate on high- and low-glucosinolate lines of *B. juncea* and *S. alba* did not differ (Bodnaryk, 1997). In contrast, the feeding rate of generalist bertha armyworm, *Mamestra configurata* Wlk. (Lepidoptera: Noctuidae), was higher on selected low glucosinolate lines of *B. juncea* and *S. alba* than their high glucosinolate parental lines (Bodnaryk, 1997). These observations lead to the implication that the development and release of low glucosinolate varieties of crop plants may not help to manage *P. xylostella*, and such varieties will also likely become more susceptible to certain generalist insect pests.

1.3.1.1.2 Glucosinolate-Myrosinase: A 'Mustard Oil Bomb'

Upon tissue disruption (e.g. by herbivory), glucosinolates undergo hydrolysis, which is catalyzed by myrosinase (Mithen, 1992; Ratzka et al., 2002; Pontoppidan et al., 2005). Glucosinolates and myrosinase are separated spatially in plants either within different cells or by subcellular compartmentalization (Bones and Rossiter, 1996). Normally, glucosinolates are present in the majority of the cells while myrosinase is located in special myrosin cells (Rask et al., 2000). Myrosinase acts as a β-thioglucosidase to produce an unstable aglucone which then forms a variety of products such as isothiocyanates that may be toxic to insects (Mithen, 1992; Xue et al., 1995; Rask et al., 2000; Wallace and Eigenbrode, 2002). In *A. thaliana*, Kliebenstein et al. (2002) mapped quantitative trait loci (QTL) that controlled glucosinolates, myrosinase and host plant resistance to *P. xylostella* and cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). They reported a number of QTL that were specific to either one herbivore or the other, as well as a single QTL (*erecta* region in the L*er* x C*vi* lines) that could control resistance to both herbivores.

In Brassicaceae, the glucosinolate-myrosinase two-component system was considered a “mustard oil bomb” against crucivores (Luthy and Matile, 1984) but this “bomb” is disarmed by glucosinolate-sulfatase (GS) of *P. xylostella*. Sulfatase activity largely prevents the formation of toxic hydrolysis products (Ratzka et al., 2002). High levels of GS in the larval gut may outcompete myrosinase in two ways viz., directly by removing the myrosinase substrate (the glucosinolates), and indirectly by reducing its activity by means of the released free sulfate (Ratzka et al., 2002). GS desulfates all major classes of glucosinolates enabling *P. xylostella* herbivory on a wide range of crucifers having diverse glucosinolate structures (Ratzka et al., 2002). Higher contents of total glucosinolates in host plants do not affect *P. xylostella* herbivory because of excess quantities of GS in the larval gut (Ratzka et al., 2002). However, elevated myrosinase levels in host plants tend to decrease the feeding activities of *P. xylostella* (Thorsteinson, 1953; Li et al., 2000).

1.3.1.2 Host Plant Volatiles

Plutella xylostella adults are attracted to volatiles emanating from their host plants (Palaniswamy et al., 1986; Pivnick et al., 1990; Renwick and Radke, 1990; Hughes et al., 1997; Reddy and Guerrero, 2000; Badenes-Perez et al., 2004; Reddy et al., 2004). Reddy and Guerrero (2000) assayed cabbage leaf extracts and reported three green leaf volatiles (GLVs) viz., (Z)-3-hexenyl acetate, (E)-2-hexenal, and (Z)-3-hexene-1-ol that were attractive to gravid females. Mated females were more responsive to these GLVs than males or virgin females suggesting that GLVs attracted mated females for oviposition (Reddy and Guerrero, 2000). In free-choice tests, Reddy et al. (2004) observed that *P. xylostella* oviposition was significantly greater on cabbage (*B. oleracea* subsp. *capitata*) followed by cauliflower (*B. oleracea* subsp. *botrytis*), broccoli (*B. oleracea* subsp. *italica*) and kohlrabi (*B. oleracea* subsp. *gongylodes*). Wind tunnel studies confirmed that orientation of *P. xylostella* females toward cabbage and cauliflower was greater than toward broccoli or kohlrabi (Reddy et al., 2004). Volatile chemicals produced by the hydrolysis of glucosinolates are also known to be involved in host plant location by crucifer specialists including *P. xylostella* (Gupta and Thorsteinson, 1960b; Pivnick et al., 1994; Blight et al., 1995; Marazzi et al., 2004). Allyl isothiocyanates (also known as mustard oils), the hydrolysis products of mainly aliphatic glucosinolates, were feeding attractants for *P. xylostella* and also stimulated/enhanced oviposition (Gupta and Thorsteinson, 1960a, b; Hillyer and Thorsteinson, 1969, 1971; Renwick, 2002; van Loon et al., 2002). Moths given both volatile and contact stimuli (such as sinigrin) deposited significantly more eggs than without volatiles (Justus and Mitchell, 1996). However, high concentrations of allyl isothiocyanate were toxic to *P. xylostella* adults (Hillyer and Thorsteinson, 1969) and neonates (Li et al., 2000).

1.3.1.3 Other Compounds

High pressure liquid chromatography revealed that the butanol extracts of wormseed mustard, *E. cheiranthoides,* contained cardenolides that were strong oviposition stimulants for *P. xylostella* (Renwick and Radke, 1990). Certain non-polar compounds that did not appear to be related to the glucosinolate group also were found to stimulate *P. xylostella* oviposition (Hughes et al., 1997). Identification of these compounds and other potential compounds in crucifers may give a new direction to *P. xylostella* behavioral studies.

Over 88 plant species, most belonging to the families Asteraceae, Fabaceae or Euphorbiaceae, have been reported to possess repellent(s) for *P. xylostella* oviposition and/or herbivory (Morallo-Rejesus, 1986). Applications of such compounds may impair *P. xylostella* oviposition even on natural host plants. For instance, the secondary plant compounds, coumarin and rutin, are known to deter *P. xylostella* oviposition (Tabashnik, 1985). They do not occur in crucifers at substantial levels but their concentrations are relatively high in many non-crucifers (Leung, 1980). A coumarin-containing legume, *Melilotus officinalis* (L.), was less acceptable for *P. xylostella* oviposition than the coumarin-free species, *Melilotus alba* (Desr.) (Gupta and Thorsteinson, 1960b). Additional studies indicated that *P. xylostella* oviposition was significantly reduced on cabbage plants treated with coumarin, rutin, and a mixture of both coumarin and rutin. The effects of coumarin and rutin were dose-dependent; rutin was a comparatively weak deterrent at a concentration of 0.01M. In choice tests, plants sprayed with the mixture of coumarin and rutin received 1.6-fold fewer eggs than coumarin-treated plants and 1.9-fold fewer eggs than rutin-treated plants (Tabashnik, 1985). Two drimane terpenoids, the sesquiterpenoid polygodial and the neem extract Margosan-O exerted significant inhibitory effects on oviposition by *P. xylostella* (Qiu et al., 1998). The plant extracts of *Azadirachta indica* A. Juss. (Meliaceae), *Acorus calamus* L. (Araceae) and *Melia azedarach* L. (Meliaceae) also deterred *P. xylostella* oviposition 24 h after treatment (50.3%, 40.5% and 39.5%, respectively) (Patil and Goud, 2003). These and other similar compounds can prove effective for reducing pest populations particularly when, after appropriate research, they are suitably integrated with the available pest management tactics.

Plutella xylostella is sensitive to certain compounds such as resin glycoside from the periderm of storage roots from sweet potato, *Ipomea batates* (L.) Lam. (Convolvulaceae). Larval survival and development declined significantly on resin glycoside-treated diet, and lifetime fecundity of adults that developed from larvae fed on treated diet also was negatively affected (Jackson and Peterson, 2000). In greenhouse studies, aqueous emulsion of an ethanolic seed extract (0.5%) of *Annona squamosa* L. (Annonaceae) was 2.5-fold more effective against *P. xylostella* larvae than 1.0% rotenone and efficacy of crude seed extracts was comparable to pyrethrum (Leatemia and Isman, 2004).

1.3.2 Morphological Bases of Resistance/Susceptibility

Morphological bases of resistance can be classified as remote factors, plant architecture/anatomical features and surface factors according to the level at which they function against the insect. Remote factors (e.g. leaf color) and plant architecture influence the orientation of the insect towards the plant, and hence can have an antixenotic effect. Surface factors that provide mechanisms of resistance include the presence or absence of pubescence and the presence and type of epicuticular waxes.

1.3.2.1 Leaf Color, Size and Position on Plant

Leaf color is one of the most important morphological characters found to influence host plant selection by *P. xylostella*. Glossy phenotypes generally have greener and darker leaves than their waxy counterparts and are more attractive for *P. xylostella* oviposition (see Section 1.3.2.2). Total leaf area and leaf shape do not seem to be major factors determining ovipositional preference by *P. xylostella*. Glossy and waxy collards and cabbage had similar leaf areas that were significantly different from those of Indian mustard and yellow rocket; however, ovipositional preference differed among the hosts with similar leaf areas. In multiple choice tests, numbers of eggs deposited on glossy collards, yellow rocket, and Indian mustard were 3, 12, and 18 times higher, respectively, than on cabbage, (Badenes-Perez et al., 2004). Egg numbers correlated negatively with leaf position from the outermost to the innermost leaves of Chinese cabbage; females laid six to eight times more eggs on outer leaves than on inner leaves (Talekar et al., 1994).

1.3.2.2 Epicuticular Waxes

Epicuticular waxes form the outermost layer of aerial plant parts (Jenks et al., 1995) and are believed to mitigate abiotic and biotic stresses including drought (Jordan et al., 1984), fungal pathogens (Jenks et al., 1994) and insect herbivores (Eigenbrode and Espelie, 1995). These waxes are comprised predominantly of long chain aliphatic lipids, but also include triterpenoids and secondary metabolites such as flavonoids and sterols (Kunst and Samuels, 2003).

Plutella xylostella prefers to oviposit on glossy (i.e., reduced surface wax) compared with waxy (i.e., normal wax bloom) cultivars of cruciferous crops (Eigenbrode et al., 1991a, b; Justus et al., 2000; Ulmer et al., 2002). Under field conditions, for example, females deposited significantly more eggs on glossy plants of *B. napus* than on their waxy counterparts (Justus et al., 2000). *Plutella xylostella* showed a significant preference for glossy collards over cabbage and laid 300 times more eggs on glossy collards than on cabbage. Similar egg numbers were laid on waxy collards and cabbage (Badenes-Perez et al., 2004). Oviposition on cabbage with normal leaf surface wax was significantly lower but increased greatly when leaf surface wax was removed with the help of a synthetic detergent (Uematsu and Sakanoshita, 1989).

Despite the fact that glossy phenotypes are attractive for oviposition, *P. xylostella* herbivory, larval survival and pupal weight were reduced on such glossy-leaved plants (Dickson and Eckenrode, 1980; Eigenbrode et al., 1990, 1991a, b; Ulmer et al., 2002; Badenes-Perez et al., 2004). Larval survival declined significantly on glossy collards compared with waxy collards (Badenes-Perez et al., 2004). Reduced survival of *P. xylostella* larvae on glossy phenotypes is associated with reduced amounts of surface wax and lower densities of wax crystallites on leaves of these plants compared with susceptible phenotypes that have a normal wax bloom (Eigenbrode et al., 1991a). Pupal weight was lower on the glossy line of *Brassica rapa* L. than on its waxy counterpart (Ulmer et al., 2002).

Plutella xylostella neonates spent more time walking and searching and less time biting on the glossy phenotypes of cabbage relative to movement rates on susceptible counterparts with normal wax, and this behavior was interpreted as indicative of reduced larval acceptance of these plants (Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991a, b). The glossy waxes have smaller proportions of n-alkanes, secondary alcohols, and ketones and greater proportions of n-alkanoic acids (fatty acids), n-alkane-1-ols (primary alcohols), and triterpenoids compared with typical *B. oleracea* waxes (Eigenbrode et al., 1991b). The components comprising a greater proportion of waxes from the susceptible crucifers may include stimulants that increase biting and palpating and decrease walking, while those comprising a greater proportion in waxes from resistant glossy plants may include deterrents that reduce acceptance behavior by the larvae (Eigenbrode and Pillai, 1998). For example, adding a mixture of four n-alkane-1-ols or a mixture of α- and β–amyrins to wax from susceptible cabbage reduced the neonate biting time and increased the walking time in a dose-dependent manner. Among individual n-alkane-1-ols, adding C_{25} alcohol reduced the time spent biting. Adding a mixture of five n-alkanoic acids did not affect biting, but increased the time spent palpating and decreased walking time. Among individual n-alkanoic acids, only adding C_{14} significantly increased the time palpating (Eigenbrode and Pillai, 1998).

Some waxes which may be inactive alone are known to synergise *P. xylostella* oviposition response to sinigrin (Spencer, 1996; Spencer et al., 1999). Certain flavonoids may stimulate the taste receptors of *P. xylostella* larvae or synergize the feeding stimulation caused in response to glucosinolates. Leaf discs of *P. sativum* treated with a mixture of flavonoids and sinigrin were significantly preferred by larvae over sinigrin-treated leaf discs alone (van Loon et al., 2002).

1.3.2.3 Pubescence

Pubescence can affect the oviposition, locomotion, herbivory and developmental time of *P. xylostella*. A significant positive correlation was observed between the number of eggs laid and trichome density (Talekar et al., 1994), suggesting that plant varieties with glabrous leaves were more resistant to *P. xylostella* oviposition (see Chapter 3). Broad bean leaves were unacceptable to neonates even after sinigrin treatment and this was associated with the physical characteristic of leaves that prevented neonate feeding (Gupta and Thorsteinson, 1960a). Barker et al. (2004) reported that developmental time of female *P. xylostella* larvae was longer on an *A. thaliana* line with trichomes (Col-0) compared with a line that lacked trichomes due to the gl1-1 mutant allele (Col-5). Adults that developed from larvae on Col-0 laid significantly fewer eggs than those that developed on Col-5 (Barker et al., 2004).

1.4 Nutritional Quality of Plants

Various competing hypotheses have been developed to explain how bottom-up forces (resources) affect herbivory on plants. For instance, the Plant Stress Hypothesis of White (1969) proposes that, when physiologically stressed, plants become more susceptible to herbivores because of reduced protein synthesis and increased amino acids in tissues, thus generating a more nutritious food for nitrogen-limited organisms. In contrast, the Plant Vigour Hypothesis of Price (1991) predicts that many herbivore species feed preferentially on vigorous plants or plant modules. Empirical evidence exists in support of both hypotheses. Some studies have shown that insects perform better on stressed plants (White, 1969, 1984; Mattson, 1980; Meyer, 2000) while others show that plants growing under fertile conditions frequently support higher densities of insect herbivores than plants growing under less fertile conditions (Fox et al., 1990; Price, 1991; Meyer and Root, 1996; Dosdall et al., 2004a). Thus soil fertility levels can either ameliorate or exacerbate oviposition and herbivory in *P. xylostella* (see Chapter 2). The host/site selection for oviposition may be influenced by the level of nutrients present in leaves and differences in food-plant quality may affect life history parameters such as survival and development of insects, directly relevant to population dynamics (Bjorkman, 2000; de Bruyn et al., 2002).

Fraenkel (1959) suggested that secondary plant compounds are cues to relative nutritional acceptability. This is most likely the case for *P. xylostella* because *Brassica* species produce sulfur-containing glucosinolates, and plant vigor is greater when sulfur supply is not limiting (Dosdall et al., 2004a). For instance, *P. xylostella* oviposition was significantly higher on sulfur-fertilized *Brassica* species than on sulfur-deficient controls (Gupta and Thorsteinson, 1960b). Similarly, numbers of eggs laid on artificial leaves treated with the wax-free methanolic leaf-surface extracts of plants grown under normal sulfur (S_n, field concentration) were significantly higher (ca. 250%) than on artificial leaves sprayed with sulfur-free (S_0) plant extracts (Marazzi et al., 2004).

1.5 Bt- transgenic Crucifers: Potential and Strategies for *P. xylostella* Management

Bacillus thuringiensis (*Bt*) Berliner-based products have been used for over 45 years as insecticidal sprays, and *P. xylostella* is the first, and still the only species, to develop resistance to this bacterial insecticide in the field (Tabashnik et al., 2003; Heckel et al., 2004; Sarfraz, 2004; Sarfraz and Keddie, 2005). Its resistance to Bt-foliar sprays has been reported from several countries including the U.S.A. (Tabashnik et al., 1990; Shelton et al., 1993), the Philippines (Ferré et al., 1991), Japan (Morishita et al., 1992), Malaysia (Verkerk and Wright, 1997), Central America (Pérez and Shelton, 1997), Mexico (Díaz-Gomez et al., 2000) and India (Mohan and Gujar, 2003b). Bt-transgenic plants are now being tested for their use both as field and trap crops for *P. xylostella* management (also see Section 1.7).

Bt-transgenic plants effectively control *P. xylostella* (Ramachandran et al., 1998b), and at present there is no evidence for insect resistance to Bt-plants in the field. Research to date suggests that Bt-plants may, in fact, be more effective for managing *P. xylostella* than Bt-foliar sprays because Bt-toxin concentrations can be regulated more effectively in transgenic plants than with sprays, and *Cry* toxins can be programmed for expression only in specific plant stages or structures. However, certain laboratory studies have shown that *P. xylostella* has the ability to develop resistance to Bt-plants expressing high levels of *Cry1Ac* (Metz et al., 1995) and *Cry1C* (Zhao et al., 2000). Several strategies have been proposed to delay the evolution of resistance, but the only commercially available approach is the use of a high dose of a single gene, producing 25 times the toxin concentrations needed to kill susceptible individuals, in combination with refugia (Shelton and Zhao, 2000). The refugia are composed of non-transgenic plants and are intended to generate sufficient numbers of susceptible insects to dilute resistant alleles in *P. xylostella* population. There is a considerable debate on the required size and spatial placement of refugia. Presently, a refuge comprising 20% of the cropping area is recommended for cotton and corn, but some workers have called for refugia as large as 50%, if farmers are allowed to spray them. Optimal refuge sizes and their placement within crops are under investigation for Bt-crucifers. In field tests, the cumulative number of *P. xylostella* larvae per plant on refugia through the season in the 20% mixed refuge was significantly lower than the 20% separate refuge (Shelton et al., 2000b). In a simulation study, Cerda and Wright (2004) tested five different refuge sizes (5 to 50% of the total cropping area) in four different spatial patterns inside the crop (border, central, equidistant and random). They showed that the rate of increase in the frequency of resistance declined with the increase in refuge size, and positioning refugia in a border resulted in a lower rate of increase in resistance frequency even when the refugium was less than 10% of the total area cropped.

The simulation study and field tests to date suggest that separate refugia comprising 15 to 20% of the crop area are more effective than the randomly mixed refuge as it likely better deters movement of larvae from non-transgenic to transgenic plants and *vice versa*. In addition, *P. xylostella* are more likely to survive to adults on refuge plants that are well separated spatially from resistant transgenic plants, enabling them to mate with resistant individuals and so lower the frequency of resistance alleles in the population. The strategy of complementary insecticidal spray applications to *P. xylostella* in refugia is not appropriate for resistance management because the main purpose of refuge plants is to generate susceptible individuals. Further, *P. xylostella* may become resistant to the insecticides sprayed in refugia, complicating the management of insect resistance to Bt-plants. However, certain selective insecticides and/or botanical oviposition deterrents (see Section 1.3.1.3) could be applied on Bt-crops resulting in reduced *P. xylostella* infestations and thus generating fewer individuals that would be exposed to Bt-toxins.

The potential of pyramiding genes is also being investigated for management of *P. xylostella* resistance to Bt-plants. This strategy involves combining two or more dissimilar major resistance genes in a single variety and thus reducing the possibility of a pest insect assembling the right combination of virulence genes to be able to attack the variety. Laboratory studies indicated that broccoli and collard plants engineered with both *cry1Ac* and *cry1C* genes effectively control *P. xylostella* populations resistant to one or the other toxin (Cao et al., 2002, 2005; Zhao et al., 2003, 2005) and thus have the potential to delay the evolution of *P. xylostella* resistance to Bt-crops.

1.6 Conventional and 'Dead-End' Trap Cropping

Trap crops, important components of cultural control, are composed of one or more plant species grown to attract a pest species in order to protect a nearby main crop (Hokanen, 1991). Protection may be achieved by preventing the pest from reaching the crop, or by concentrating the pest in a portion of the field where it can be managed (Shelton and Nault, 2004). In some cases, trap crops may serve as a habitat for natural enemies that can then increase and suppress the pest populations (Zhao et al., 1991). In other cases, suitable insecticides or cultural practices such as destroying the trap crop could be deployed (Shelton and Nault, 2004). The two most commonly proposed trap crops for *P. xylostella*, Indian mustard (*B. juncea*) and collards (*B. oleracea* var. *acephala*), have been determined to be unreliable (Silva-Krott et al., 1995; Luther et al., 1996; Bender et al., 1999; Charleston and Kfir, 2000; Meena and Lal, 2002; Shelton and Nault, 2004) (see Chapter 3).

Recently, Shelton and Nault (2004) suggested a 'dead-end' trap cropping strategy as a new approach for *P. xylostella* management. This strategy utilizes a plant species such as glossy yellow rocket that is highly attractive for oviposition but on which *P. xylostella* larvae cannot survive (Idris and Grafius, 1996; Badenes-Perez et al., 2004;

Shelton and Nault, 2004). *Plutella xylostella* larvae refused to feed on glossy (G)-type yellow rocket, *B. vulgaris* var. *arcuata*, and failed to survive on this wild crucifer (Idris and Grafius, 1996; Shinoda et al., 2002; Agerbirk et al., 2003; Badenes-Perez et al., 2004) while the pubescent (P)-type was susceptible to *P. xylostella* (Agerbirk et al., 2003). The antibiotic activity of G-type yellow rocket is attributed primarily to saponins. For example, all neonates died on cabbage leaf discs treated with a monodesmosidic triterpenoid saponin (3-O-[O-β-D-glucopyranosyl-(1→ 4)-β-D-glucopyranosyl]-hedragenin) at concentrations comparable to those in fresh leaves of *B. vulgaris* (>0.18 μg mm^2), and leaf consumption by third instars was greatly reduced (<11% of controls) (Shinoda et al., 2002). Another triterpenoid saponin, 3-O-β-cellobiosyloleanolic acid from foliage of G-type yellow rocket, also inhibited *P. xylostella* larval growth (Agerbirk et al., 2003). First-instar larvae of *M. configurata* never reached the second-instar on pennycress, *Thlaspi arvense* L., and the antibiotic activity was associated with the presence of saponins (Dosdall and Ulmer, 2004). However, *P. xylostella* can develop successfully on pennycress (Idris and Grafius, 1996) suggesting that saponin profile may be different in yellow rocket and pennycress or some additional factors of the host plant and/or the herbivore contribute to this activity. In free-choice tests, the number of eggs laid on yellow rocket was 12 times greater than on cabbage (Badenes-Perez et al., 2004). In the laboratory, *P. xylostella* laid 5.5-fold more eggs on *B. vulgaris* than on *B. napus* subsp. *oleifera*. About 92% of the larvae reared on *B. napus* survived after 96 and 120 h, whereas significantly fewer larvae survived on *B. vulgaris* after 96 h and none survived after 120 h. In outdoor screenhouse studies, *P. xylostella* laid 24 to 66 times more eggs on

B. vulgaris compared with cabbage or broccoli and again no larvae survived on *B. vulgaris* (Shelton and Nault, 2004).

Plutella xylostella was also very sensitive to ingestion of mustard trypsin inhibitors. For example, larvae could not survive on *A. thaliana* plants expressing mustard trypsin inhibitors at 0.6 to 0.8% of soluble proteins. A lower expression level of this inhibitor (0.2 to 0.3%) as observed in oilseed rape lines (1B, 2B) caused high mortality (57% and 33%, respectively) and also delayed development of *P. xylostella* (Leo et al., 2001). These findings suggest that the trap plants that express high levels of mustard-trypsin inhibitors can prove dead-end hosts for *P. xylostella* while low concentrations of these inhibitors in transgenic main crops may also partially confer resistance to this pest insect.

1.7 Host-Plant Interactions: Implications for *P. xylostella* Management

Insect-resistant plant varieties are important tools for integrated pest management programs. van Emden (1991) argued that insecticide concentrations could be reduced three-fold on resistant host plants without appreciable increases in the pest population. To date, the most successful efforts directed toward producing varieties of brassicaceous crops with resistance to *P. xylostella* have been through development of transgenic crops modified to express the Bt-toxin (Cao et al., 2002, 2005; Zhao et al., 2003, 2005). However, strategies for developing resistance by modifying biochemical and morphological plant characteristics have been incompletely exploited, in spite of their potential effectiveness for prolonging the usefulness of Bt technology.

Perhaps the most compelling reason why intensive plant breeding efforts have not been directed toward non-Bt transgenic approaches for resistance is because it appears likely that *P. xylostella* utilizes an integrated suite of chemical and morphological cues for host plant location and recognition. Developing resistant germplasm for multiple traits, likely with complex inheritance patterns, is a daunting task, accompanied with the risk of rendering the host plant more susceptible to generalist insect herbivores. Glucosinolates, cardenolides, plant volatiles, waxes, plant nutritional quality, leaf morphology and leaf color, or a combination of these factors, may trigger reproductive and feeding activities of *P. xylostella*. Several researchers suggest that glucosinolates in Brassicaceae play a major role in plant defensive mechanisms against generalists and attraction to specialist insect herbivores (e.g. Thorsteinson, 1953; Blau et al., 1978; Reed et al., 1989; Bodnaryk, 1997; Li et al., 2000; Ratzka et al., 2002). However, the influence of glucosinolates on the interactions of herbivores with their host plants should be examined on a case-by-case basis (Blau et al., 1978), through assessments of the types and total concentrations of glucosinolates and their metabolites, and the developmental stage of the plant and tissue(s) being fed upon (Bodnaryk and Palaniswamy, 1990; Bodnaryk, 1991, 1997). Plant breeding programs aimed specifically at glucosinolate reduction have made available brassicaceous genotypes that differ in foliar glucosinolate concentrations from their parental lines permitting detection and assessment of resistance mechanisms that are independent of glucosinolates (e.g. morphological plant characteristics). For example, of two low-glucosinolate lines of *B. juncea* evaluated by Bodnaryk (1997), one was resistant to *P. xylostella* herbivory compared with the second, suggesting that other mechanisms were involved in this resistance. To date, insect physiologists and chemical ecologists have not been completely successful in identifying the specific biochemical and morphological changes necessary to convert brassicaceous hosts of *P. xylostella* into non-hosts, depriving geneticists of a firm target in their plant breeding programs.

Nevertheless, some aspects of host plant resistance can be integrated with cultural strategies for more effective management of *P. xylostella*. For example, Brassicaceae with a glossy phylloplane are not only attractive for *P. xylostella* oviposition, but the glossy trait also negatively affects larval survival and development suggesting that selected glossy cultivars have potential usefulness as trap crops in brassicaceous vegetable fields. Glossy collards may be an acceptable trap crop because it is also a preferred host for *P. xylostella* oviposition over cabbage (Badenes-Perez et al., 2004) and larval survival is much lower on it than on cabbage (Eigenbrode and Shelton, 1990). However, the susceptibility of glossy collards to crucifer flea beetles (Stoner, 1990; Bodnaryk, 1992) may decrease its effectiveness as a trap crop in areas where flea beetle population densities are high (Badenes-Perez et al., 2004). Glossy yellow rocket is a potential candidate for trap cropping. *Plutella xylostella* ovipositional preference was much greater on *B. vulgaris* var. *arcuata* than on cabbage and canola but larvae failed to survive on it (Shelton and Nault, 2004). Besides its lethality to *P. xylostella*, yellow rocket is also resistant to other major crucivores including flea beetles, *P. cruciferae*, *Phyllotreta striolata* (F.) (Badenes-Perez et al., 2004), *Phyllotreta nemorum* L. (Agerbirk et al., 2001), and the cabbage white butterfly, *Pieris rapae oleracea* (Harris) (Renwick, 2002). These discoveries should stimulate new interest in trap cropping but there remain questions to be addressed before yellow rocket could be recommended for extensive field use. For example, how well does *B. vulgaris* compete with the main field crops it is used to protect, particularly brassicaceous vegetables, and what are its ultimate effects on crop quality and quantity? How should *B. vulgaris* be placed spatially on farms to reduce the likelihood of competition and yet serve as an effective trap crop? Does *B. vulgaris* grow equally well worldwide wherever brassicaceous crops are grown? Would *B. vulgaris* contribute detrimentally to the weed seed bank in soils? Research to address these questions should lead to appropriate implementation of this promising new development in integrated management of *P. xylostella*.

Other options exist that can make *P. xylostella* management more effective. As discussed earlier, glossy phenotypes are more attractive for *P. xylostella* oviposition and ovipositing females do not discriminate among Bt-transgenic and wild-type plants. However, neonates have high mortality on transgenic plants suggesting that glossy phenotypes supplemented with Bt-toxins may serve as promising tools for dead-end trap cropping. For example, in detached leaf assays, ovipositional preference by *P. xylostella* on Bt-collards expressing *Cry1C* and wild-type collards did not differ significantly and no larvae could survive on leaves from Bt-plants (Cao et al., 2005). Similarly, both Bt-resistant and Bt-susceptible strains of *P. xylostella* did not discriminate for oviposition on Bt-transgenic and wild-type canola plants. Bt-canola killed all larvae tested from the Bt-susceptible *P. xylostella* strain (Ramachandran et al., 1998a). Additional studies determined that *P. xylostella* did not discriminate between Bt-transgenic and wild-type broccoli. Survival of susceptible second-instar larvae on *Cry1Ac*-expressing broccoli declined from 99.1 to 19.2% at 24 and 72 h, respectively, and larval weight gain was 0.2 mg/10 larvae at 24 h (Tang et al., 1999). In the laboratory, after hatching on Bt-cabbage expressing *Cry1Ab*, *P. xylostella* neonates had 100% mortality and did not move to non-transgenic plants placed in the vicinity (Kumar, 2004). The author speculated that neonates perceived identical sensory signals from the transgenic and wild-type plants. These findings suggest that Bt-plants do not have significant antixenotic effects on *P. xylostella* and hence there would be less likelihood of emigration of insects from Bt-trap crops to non-transgenic field crops in the vicinity.

The gene(s) responsible for saponins, mustard trypsin inhibitors, glucosinolate-sulfatse inhibitors, and other toxicants may be considered for their expression in acceptable trap crops. Oviposition deterrents or repellents require further investigations for field use. Crucifer growers may manipulate fertilizer applications to manage *P. xylostella* (Chapter 2) and other crucivores such as root maggots (Dosdall et al., 2004a). Intercropping with non-crucifers can also minimize *P. xylostella* infestations (Buranday and Raros, 1975; Talekar et al., 1986; Meena and Lal, 2002; González-Rodríguez and Macchiavelli, 2003; Bukovinszky et al., 2005). In addition to selection of an effective companion crop, it is also essential to determine an appropriate intercropping system on the basis of modeling spatial and temporal patterns affecting *P. xylostella* dispersal within and between crops (Cameron et al., 2002; Mo et al., 2004) (see Chapter 8), and possible competition between the companion crop and the main crop.

Part- II: Biological Control of Diamondback Moth

1.8 Natural Enemies: Predators and Parasitoids

In an insightful statement, Marsh (1917) outlined the pest status of *P. xylostella* in the U.S.A. and declared, "The diamondback moth is a striking example of a potentially serious pest normally held in repression by parasites." On a worldwide scale, over 135 parasitoid species have been recorded to attack various stages of *P. xylostella* (Delvare, 2004), with the most common ones comprising six species of egg parasitoids, 38 larval, and 13 pupal parasitoids (Lim, 1986; Talekar and Shelton, 1993) (Fig. 1). Certain ants, flies, lacewings, hemipterans, beetles, spiders and birds also prey on its larvae (Alam, 1992; Anonymous, 1996; Reddy et al., 2004; Endersby and Cameron, 2004). Some predators such as *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) were attracted to *P. xylostella* pheromone blend, larval frass and green leaf volatiles of crucifers (Reddy et al., 2004). These generalist predators have not usually been considered significant factors in regulation of *P. xylostella* populations although some ants such as *Anomma nigricans* (Illiger) (Hymenoptera: Formicidae) are major control agents of *P. xylostella* in periurban areas of Benin, West Africa (Goudegnon et al., 2004). Egg parasitoids (genera *Trichogramma* and *Trichogrammatoidea*) (Hymenoptera: Trichogrammatidae) do not exert adequate control, as they require frequent mass releases (Talekar and Shelton, 1993). In addition, egg parasitoids are not always host-specific (Goulet and Huber, 1993) and may pose a threat to non-target species in a region. For example, *Trichogramma brassicae* Bezdenko, which was inundatively released against the European corn borer in Switzerland, parasitized eggs of 22 out of 23 lepidopteran species tested, including those on the Swiss list of endangered species (Babendreier et al., 2003a, b). Larval parasitoids have the greatest control potential and Lim (1986) suggested that the most effective belong to the hymenopteran genera *Microplitis* (Braconidae), *Cotesia* (Braconidae), and *Diadegma* (Ichneumonidae). A few prepupal and pupal parasitoids of the genus *Diadromus* (Ichneumonidae) also contribute to *P. xylostella* control (Table 1-IV) (Hardy, 1938; Liu et al., 2000; Kirk et al., 2004), and occasionally *Pteromalus* (Pteromalidae) species also parasitize *P. xylostella* pupae (Chauhan and Sharma, 2004).

Mustata (1992) reported 25 parasitoid species from Moldavia (Romania) parasitising up to 80 to 90% of *P. xylostella* populations but some of them were misidentified (see Delvare, 2004) and other records were rejected by Noyes (1994) as they were based on inaccurate data. Kirk et al. (2004) recorded 27 primary parasitoid species mainly of the genera *Diadegma, Cotesia* and *Oomyzus* on 115 populations of *P. xylostella* collected in 32 countries. In Ethiopia, *Diadegma* species and *Cotesia plutellae* (Kurdjumov) were responsible for over 90% of observed parasitism (Ayalew et al., 2004). *Cotesia plutellae* and *Oomyzus sokolowskii* (Kurdjumov) (=*Tetrastichus sokolowskii)* (Hymenoptera: Eulophidae) are considered the dominant parasitoids in China (Liu et al., 2000). *Diadegma* spp. and *Diadromus* spp. dominate in Europe (Hardy, 1938), South Africa (Kfir, 1997) and New Zealand (Todd, 1959). *Diadegma insulare* (Cresson) caused about 71% parasitism in California (Oatman and Planter, 1979). *Diadegma semiclausum* (Hellén), *D. rapi* (Cameron) and *Diadromus collaris* (Gravenhorst) collectively accounted for 93% parasitism in Victoria, Australia (Goodwin, 1979). Seven species were observed in Brasilia with *Diadegma leonitiniae* (Brethés) and *Apanteles piceotrichosus* (Blanchard) as dominant parasitoids but the total parasitisim was insufficient to regulate *P. xylostella* populations (Guilloux et al., 2004). In the Eastern Cape (South Africa), four species viz., *Diadegma mollipla* (Holmgren), *C. plutellae*, *D. collaris* and *O. sokolowskii* were major parasitoids and *P. xylostella* parasitism rates varied throughout the year ranging 10 to 80% (Smith and Villet, 2004). Shelton et al. (2002) collected 2,815 larvae and pupae of *P. xylostella* in New York from 1979 to 1994 and observed that 46.5% of them were parasitized by *D. insulare*, 7.0% by *Microplitis plutellae* (Muesebeck), 2.1% by *O. sokolowskii*, and 0.4% by *D. subtilicornis*. In contrast, the absence of effective parasitoids in areas including Southeast Asia, the Pacific islands, the Caribbean, and Central America can result in intensive *P. xylostella* infestations (Talekar and Shelton, 1993).

Canada has three major hymenopterous parasitoids of *P. xylostella*. *Diadegma insulare* and *M. plutellae* are larval parasitoids while *Diadromus subtilicornis* (Gravenhorst) attacks the prepupal and pupal stages (Harcourt, 1986; Anonymous, 1996; Braun et al., 2004). In Alberta, *D. insulare* was the principal parasitoid of *P. xylostella* in 1992, accounting for approximately 45% of the total parasitism, while *M. plutellae* and *D. subtilicornis* each accounted for about 14% of the total parasitism. A similar situation was observed in Saskatchewan with 30% parasitism by *D. insulare* and less than 8% each by *M. plutellae* and *D. subtilicornis* (Braun et al., 2004).

1.8.1 Larval Parasitoids

1.8.1.1 *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae)

Diadegma species are the principal regulators of *P. xylostella* worldwide (Table 1-V) (Marsh, 1917; Hardy, 1938; Todd, 1959; Mustata, 1992; Azidah et al., 2000, Delvare, 2004). This large genus of Campoleginae comprises about 200 species worldwide (Yu and Horstmann, 1997), with the majority (about 118 species) being described from temperate regions (Delvare, 2004).

Diadegma insulare is a solitary, host-specific larval endoparasitoid of *P. xylostella* and is one of its most important biocontrol agents in the Nearctic to the northern Neotropical regions (Harcourt, 1960, 1986; Fitton and Walker, 1992; Mukenfuss et al., 1992; Idris and Grafius, 2001). On average, it can parasitize 70 to 90% of *P. xylostella* larvae and parasitized larvae consume 35 to 80% less food than nonparasitized larvae (Mukenfuss et al., 1992; Mitchell et al., 1997; Sourakov and Mitchell, 2000; Monnerat et al., 2002). In North America, parasitism by *D. insulare* sometimes exceeds 80% for fourth instars and 50% for third instars (Hutchison et al., 2004). In south Texas, it has been reported to account for more than 98% parasitism in the field (Legaspi et al., 2000). Compared with other parasitoids, it is an efficient host searcher (Xu et al., 2001a; Wang and Keller, 2002) and has a significant ability to discriminate previously parasitized hosts (i.e. it avoids multiparasitism and superparasitism) (Bolter and Laing, 1983). During a search immediately following contact by a parasitoid, the *P. xylostella* larva typically moves away wriggling vigorously and drops from the plant suspended by a silken thread. A wasp may attempt a quick jab with its ovipositor and sometimes inserts an egg successfully. Interestingly, *D. insulare* exhibits a flexible behavior sitting motionless near the silken thread, waiting for the suspended larva to climb up and then attacks it again. Sometimes the wasp also follows down the silken thread and stings the suspended larva (Sarfraz, personal observation). A similar behavior was reported for its congeneric species, *D. semiclausum* (Wang and Keller, 2002).

Attempts have been made to rear *D. insulare* in captivity on *P. xylostella* larvae reared on artificial diet (Sieglaff et al., 1998; Johanowicz and Mitchell, 2000b) but to date no significant success has been achieved. Mass rearing presents many challenges. In the absence of a host plant (or leaf), female wasps rarely parasitize *P. xylostella* larvae (Sourakov and Mitchell, 2000). Under laboratory conditions, the ratio of males to females is usually extremely high (about 10:1) due to frequent haploid parthenogenesis; in the field this ratio is equivalent (about 1.1:1.0) (Harcourt, 1960). The proportion of male wasps from young parasitized *P. xylostella* larvae (L_2) is greater than females while larvae parasitized at L_3 and L_4 stages yield more females (Fox et al., 1990; Monnerat *et al.*, 2002). Temperature also affects parasitism rates and sex ratio. Monnerat et al. (2002) demonstrated that *Diadegma* sp. did not parasitize at 15°C, while at temperatures of 20°C, 25°C and 29°C, parasitism rates were similar. Sex ratios were identical at 20°C and 25°C but at 29°C the ratio was 3:1 (m:f).

Diadegma insulare has been reared successfully in the greenhouse with a parasitism rate of 95% with 45 to 63% females (Xu and Shelton, 2001). Sourakov and Mitchell (2002) developed a technique to achieve a higher female to male sex ratio and increased progeny. They offered more than 100 third-instar *P. xylostella* larvae to a single mated female wasp in the presence of collard leaves. Every 24 hours this female parasitoid was transferred to another container with a new group of larvae for four consecutive days. The female progeny increased from 22.6±5.8% (day one) to 59.1±5.06% (day four). They suggested that oviposition should be allowed for at least four days in order to get a higher female: male ratio and increased total numbers of progeny.

In the field, this highly mobile wasp prefers to visit or remain longer in habitats with abundant food sources and refugia for itself and its host (Idris and Grafius, 2001). Depending upon the food source, the number of *P. xylostella* larvae parasitized by a single wasp may vary from zero to 150 (Grafius, 1997). Many flowering plants provide good nectar sources for parasitoids in the field. Sweet alyssum, *Lobularia maritime* (L.) Desv. (Brassicaceae), for example, can be a good food source for augmentively released parasitoids in the cabbage agroecosystem (Johanowicz and Mitchell, 2000a).

Laboratory studies indicated that *D. insulare* can increase its tolerance to certain pyrethroids such as permethrin (Xu et al., 2001b) but the development of resistance was much slower than its host, *P. xylostella*, and its ability to build up tolerance was limited (Shelton, 2004). For instance, a *P. xylostella* population collected from California exhibited over 200-fold tolerance to permethrin (Shelton et al., 2000a) while *D. insulare* developed only a 5-fold resistance to this pyrethroid in the laboratory (Xu et al., 2001b).

1.8.1.2 *Microplitis plutellae* (Muesebeck) (Hymenoptera: Braconidae)

Microplitis plutellae is also a primary larval endoparasitoid of *P. xylostella* especially in North America. Initially it was listed only from Iowa, Colorado, Idaho, and California but now it has also been recorded from Utah, South Carolina, New York, Alberta, Saskatchewan, Ontario (Harcourt, 1960; Braun et al., 2004), Taiwan, Laos and Cambodia (Kirk et al., 2004). A congeneric species, *M. mediator* (Haliday), is also known to parasitize *P. xylostella* and can be quite common in Romania (Kirk et al., 2004). *Microplitis plutellae* can overwinter as pupa in western Canada and plays an appreciable role in regulating *P. xylostella* populations early in the year (Putnam, 1978).

It parasitizes all four instars of *P. xylostella* and the mature parasitic larva emerges from the membranous area between the fourth and fifth abdominal tergites of the final-instar host and spins its own brown, oval cocoon that adheres to any available surface (e.g. stem, leaf, wall of a cage, etc.) a short distance from the dying host (Harcourt, 1960; Putnam, 1968; Gharuka et al., 2004).

1.8.1.3 Comparison between *D. insulare* and *M. plutellae*

Although both *D. insulare* and *M. plutellae* parasitize all four instars of *P. xylostella* (with very low parasitism of first instars), *M. plutellae* kills and emerges from fourth instars, whereas *D. insulare* kills and emerges from the prepupal stage and spins its own cocoon inside the loosely woven cocoon of its host (Harcourt, 1960; Putnam, 1968). The number of generations per year of both parasitoids corresponds to the number of generations of *P. xylostella* as one host larva supports only one parasitoid larva (Sourakov and Mitchell, 2000). *Microplitis plutellae* can enter pupal diapause and successfully withstand low temperatures (0°C for 160 days) (Putnam, 1978). In contrast, cocoons of *D. insulare* completely lose their viability within 49 days at 4°C (Okine *et al.*, 1996; Sourakov and Mitchell, 2000). On average, adult *M. plutellae* live for 20 days and produce 316 eggs per female whereas adult *D. insulare* live for 26 days and lay 814 eggs per female at 23°C. The number of eggs produced per day is similar for both parasitoids, but adult *M. plutellae* have a shorter lifetime, resulting in higher overall fecundity for *D. insulare* (Bolter and Laing, 1983).

In Geneva (New York), the average parasitism of *P. xylostella* collectively by both parasitoids (*D. insulare* and *M. plutellae*) was 33.6% and 53.6% for the third and fourth instars, respectively during the cabbage-growing season (from 7 June to 18 October) (Xu et al., 2001a). For third instars, 64.9% of the total parasitism was caused by *D. insulare* and 33.0% by *M. plutellae*. For fourth instars, *D. insulare* accounted for 56.5% of total parasitism whereas 41.7% was due to *M. plutellae*. During a naive wasp test, *D. insulare* was much more active than *M. plutellae*. Of tested naive wasps, 62.5% of *D. insulare* landed on the host plants within the 20 minute observation period in contrast to 21.2% of *M. plutellae*. For experienced wasps, the difference between species was also highly significant, with 72.5% of experienced *D. insulare* and 40.0% of *M. plutellae* landing on host plants. Based on these results, Xu et al. (2001a) suggested that *D. insulare* may be more suitable for field release to augment biocontrol against *P. xylostella*.

1.8.1.4 *Cotesia (=Apanteles) plutellae* (Kurdjumov) (Hymenoptera: Braconidae)

Cotesia plutellae is an oligophagous solitary larval endoparasitoid that parasitizes a small percentage of the larval population of *P. xylostella* (Lloyd, 1940). This small braconid is distributed throughout Europe (Lloyd, 1940) and has also been reported from other parts of the world including China (Liu et al., 2000), South Africa (Kfir, 1997), Japan (Noda et al., 1996), Pakistan (Mushtaq and Mohyuddin, 1987), India (Joshi and Sharma, 1974), and Indonesia (Wilkinson, 1931). It has been introduced from Europe to several countries, including Australia, Dominica, Fiji, Thailand and the United States (Waterhouse and Norris, 1987) and from South Africa to St. Helena (Kfir and Thomas, 2001). In Pakistan, it is distributed from an altitude of 5 m (Karachi) to 1,660 m (Quetta) with highest parasitism (57.2%) of *P. xylostella* recorded in turnip fields in Hyderabad

(30 m altitude) (Mushtaq and Mohyuddin, 1987). In Hangzhou (China) it is a major parasitoid of *P. xylostella*, and remains active throughout the year, but with very low populations in winters (Liu et al., 2000).

Cotesia plutellae shows a preference for unparasitized host larvae but has poor interspecific discrimination. When no unparasitized hosts are available it readily oviposits in hosts containing larvae of other parasitoids such as *Diadegma* species (i.e. multiparasitism) (Lloyd, 1940). Velasco (1982) reported that *C. plutellae* did not parasitize fourth-instar larvae but later reports indicated that it parasitized larvae of all instars (preferably L_2 and L_3). The fourth-instar larvae parasitized by *C. plutellae* exhibit longer development times (123.8±1.8 hrs) than unparasitized ones (43.2±1.1 hrs). In contrast to *D. insulare*, larvae parasitized by *C. plutellae* consume more food (118.4±2.4 mg) than the controls (96.2±4.8 mg) (Shi et al., 2002).

Plutella xylostella and *C. plutellae* share a major metabolic mechanism of resistance (i.e., elevated levels of detoxifying enzyme, the monooxygenase) to certain pyrethroids (Liu et al., 2004) and selection of parasitoid larvae inside the resistant host can accelerate development of resistance to these insecticides in larvae and adults of *C. plutellae*. For example, the parasitoids selected with the fenevalerate-susceptible hosts acquired 4.5-fold resistance after 14 selections, while those selected with the highly resistant hosts exhibited 13.6-fold resistance to this pyrethroid after 13 selections (Liu et al., 2004). In South Africa, *C. plutellae* was abundant in the fields treated mainly with pyrethroids suggesting that it may have also developed resistance to these chemicals in the open fields (Smith and Villet, 2004).

Unlike specialist parasitoids, *C. plutellae* exhibit a fixed host searching behavior, pursue the host(s) down the silk thread and spend a significantly greater proportion of time on the ground (17.7±3.6%) than *D. semiclausum* (4.7±1.1%) (Wang and Keller, 2002). Initially this wasp was assumed to be a *P. xylostella* specialist but it has been recorded from or reared on several other lepidopterans (Fitton and Walker, 1992) including a naturally occurring hybrid of *Nyctemera amica* (White) and *N. annulata* (Boisduval) (Lepidoptera: Arctiidae) (Cameron and Walker, 1997). A glasshouse host specificity test in Australia revealed that *C. plutellae* had significant preference for *N. amica* on a noxious weed ragwort, *Senecio jacobaea* L. (Compositae) in the presence of *P. xylostella* larvae on cabbage (Endersby and Cameron, 2004). This research suggests plant volatiles from non-crucifers (e.g. *S. jacobaea*) may be more attractive to the parasitoid than those from cabbage and introductions of *C. plutellae* to such regions may pose a threat to indigenous fauna.

1.8.1.5 *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae)

The chalcid, *O. sokolowskii* is a larval-pupal endoparasitoid commonly found in China (Liu et al., 2000, 2001; Mahmood et al., 2003; Shi et al., 2004), France, Romania, Senegal, Benin, Réunion, India, Pakistan, Brazil, Guadeloupe, Martinique, Turkey, South Africa and Italy (Kirk et al., 2004; Smith and Villet, 2004). It is a gregarious parasitoid and one *P. xylostella* larva can support the complete development of about 8 to 10 *O. sokolowskii* (Ooi, 1988; Wang et al., 1999). It has a broad host range and occasionally acts as a facultative hyperparasitoid as it sometimes emerged from cocoons of *C. plutellae* (Fitton and Walker, 1992; Waterhouse and Norris, 1987; Liu et al., 2000;

Mahmood et al., 2003; Kirk et al., 2004). It can parasitize hosts already containing larvae of *D. semiclausum* without any discrimination but *D. semiclausum* kills the *O. sokolowskii* immatures inside the host (Shi et al., 2004).

1.8.2 Pupal Parasitoid

1.8.2.1 *Diadromus (=Thyraeela) collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae)

Diadromus species are primary prepupal and pupal parasitoids of *P. xylostella* in various regions of the world including England (Hardy, 1938), Holland (Lloyd, 1940), Canada (Harcourt, 1960; Anonymous, 1996; Braun et al., 2004), Australia (Goodwin, 1979), Moldavia (Mustata, 1992), South Africa (Kfir, 1997, 1998; Kirk et al., 2004), China (Liu et al., 2000), India (Chauhan and Sharma, 2004), France, Turkey, Bulgaria, Georgia and Greece (Kirk et al., 2004).

Diadromus collaris is perhaps the most well known species of this genus. It is a solitary endoparasitoid that supplements the control achieved by other parasitoids. It spends its egg, larval and pupal stages inside the *P. xylostella* pupa while the adult is free-living. It has been successfully introduced and established in different regions including Australia (Wilson, 1960; Goodwin, 1979), Barbados, New Zealand (Beck and Cameron, 1990), Malaysia (Ooi and Lim, 1989) and St. Helena (Kfir and Thomas, 2001).

When there is opportunity, the *D. collaris* female prefers to parasitize pupae that are in the first half of their pupal development, but oviposition is not restricted when no other choice is available; however, its survival decreases sharply with an increase in host pupal age (Lloyd, 1940; Wang and Liu, 2002). The presence of a silken cocoon surrounding the host pupa plays an appreciable role in the acceptance and parasitization of a particular host. This species exhibits superparasitism but not multiparasitism. Lloyd (1940) offered *P. xylostella* prepupae containing advanced larvae of *D. semiclausum (=Angitia cerophaga)* to ten *D. collaris* females alternately with unparasitized prepupae. A total of 75 eggs were laid; all were placed in unparasitized hosts. Similarly, Liu et al. (2001) offered *P. xylostella* pupae that were parasitized as fourth-instar larvae by *O. sokolowskii*. The female *D. collaris* examined these host pupae by inserting its ovipositor. However, dissection of such pupae revealed that *D. collaris* females did not deposit any eggs in the host pupae already containing *O. sokolowskii* immature stages and this examination by *D. collaris* did not show any adverse effect on *O. sokolowskii* survival (Liu et al., 2001).

1.8.3 Releases of Parasitoids: Successes and Failures

If naturally occurring biocontrol agents fail to colonize infested fields, or colonize too late in the season to provide effective pest control, augmentive or inundative releases of natural enemies may be effective for reducing pest damage (Collier and Steenwyk, 2004). In contrast, inoculative releases entail the release of relatively low numbers of biocontrol agents early in the season allowing them to establish successfully for pest control later on. This strategy may prove to be an effective tool where *P. xylostella* is known to overwinter and causes predictable infestations and damage later in the season.

One of the earliest parasitoid introductions was made in New Zealand in 1935 when no significant control of *P. xylostella* by the indigenous parasitoids was evident. The climatic similarities of England and New Zealand prompted entomologists to introduce *D. semiclausum* and *D. collaris*, which are effective biocontrol agents in England, into the latter country. The introduced parasitoids currently continue to suppress the pest populations along with the native *D. novaezealandiae*, but still need to be incorporated properly into modern integrated pest management programs (Hardy, 1938; Talekar and Shelton, 1993). In the early 1950s, *D. semiclausum* was introduced from New Zealand into the highlands of Java (Indonesia) where it provided significant control of *P. xylostella* in conjunction with *Bt*-insecticides (Vos, 1953). This parasitoid was subsequently also introduced from Java to the other islands in Indonesia (Talekar and Shelton, 1993). When *C. plutellae* could not provide adequate control, *D. semiclausum* was introduced from Indonesia to Taiwan in 1985. In the lowlands, this parasitoid failed to establish due to prevailing high temperature conditions; however, in the highlands it accounted for more than 70% parasitism (AVRDC, 1986, 1988). *Microplitis plutellae* was introduced from the U.S.A. to Taiwan in 1995 (AVRDC, 1998). In 2001, the International Centre of Insect Physiology and Ecology (ICIPE) imported *D. semiclausum* from Taiwan and released it in Kenya for biocontrol of *P. xylostella* (Wagener et al., 2004b).

Three parasitoids viz., *D. semiclausum, C. plutellae* and *D. collaris* were introduced to Australia and these introductions resulted in 72 to 94% parasitism of *P. xylostella*. Among the introduced parasitoids, *D. semiclausum* became established throughout Australia, including Tasmania, *C. plutellae* established in Australian Capital Territory, New South Wales and Queensland, and *D. collaris* established mainly in Queensland, New South Wales, Victoria, and Tasmania (Wilson, 1960; Goodwin, 1979).

In 1970, *C. plutellae* obtained from India was introduced to several Caribbean countries including Grenada, St. Vincent, St. Lucia, Dominica, Antigua, Montserrat, Belize, St. Kitts-Nevis Trinidad, Barbados and Jamaica. In some release sites, the parasitoid was recovered, but it did not prove very effective in *P. xylostella* suppression. Its reintroduction to Jamaica in early 1989 resulted in its establishment; parasitism increased from 5.4% in the first generation following introduction to 88.7% by March 1990 (Alam, 1992; Talekar and Shelton, 1993).

In 1977 to 1978, Malaysian entomologists introduced *D. semiclausum, O. sokolowskii* and *D. collaris* to the Cameron Highlands where crucifers are grown year round. Although all parasitoid species were recovered the following year(s), the combined parasitism level by both *D. semiclausum* and *D. collaris* was only 6%. *Oomyzus sokolowskii* was recovered in 1978 but was not found in 1984. Surveys in 1989 revealed that *D. semiclausum* and *D. collaris* had become abundant enough to adequately suppress *P. xylostella* populations (Chua and Ooi, 1986; Ooi and Lim, 1989; Syed et al., 1990).

Plutella xylostella is a serious pest on the island of St. Helena, a small British island in the South Atlantic Ocean. *Diadegma mollipla* (Holmgren) was the only parasitoid on the island but because of extensive use of synthetic pesticides its impact was minimal. The Plant Protection Research Institute (PPRI) in Pretoria, South Africa introduced *C. plutellae* and *D. collaris* to St. Helena. A total of 17,500 *C. plutellae* and 23,500 *D. collaris* were released in 10 different farms across the island continuously from May 1999 to September 2000 and farmers were advised to replace chemical insecticides with Bt to maximize opportunities for parasitoid establishment. During January to March

2000, *P. xylostella* larvae were sampled from 16 farms and *C. plutellae* exhibited a high parasitism level (27.7 to 80%) and was present in samples from 15 farms, eight of which were not release sites. *Diadromus collaris* was also recovered from five of 14 farms with a parasitism rate as high as 55%. In 2001, the use of chemical and *Bt* insecticides declined dramatically because *P. xylostella* was suppressed by native and introduced biocontrol agents (Kfir and Thomas, 2001).

1.8.4 Population Genetics: Implications for Biological Control of *P. xylostella*

The success of biological control programs depends on accurate identification of the natural enemies and the linkage of host strain with coevolved natural enemies. Misidentifications or failure to match host and parasitoid genotype may lead to program failure and/or threat to native beneficial fauna. According to Noyes (1994) about 75% of host-parasitoid records are misleading because they are based on misidentifications either of hosts or their parasitoids. The main taxonomic dilemma concerns the genus *Diadegma* (Fitton and Walker, 1992). For instance, in 1953, *D. insulare* from Kenya was imported and released in Hawaii, but failed to establish (Johnson et al., 1988). In 1998, the *Diadegma* species from Kenya were identified as *D. semiclausum* by the Natural History Museum, London (Wagener et al., 2004b). In 2000, all African *Diadegma* species were classified as *D. mollipla* on the basis of common morphological characters (Azidah et al., 2000) (Table 1-V). Subsequently, Wagener et al. (2004b) separated seven different *Diadegma* species using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis and also discovered a new *Diadegma* species from Ethiopia. DNA from the seven *Diadegma* species was successfully amplified with the internal transcribed spacers (ITS2-1 and ITS2-2) resulting in three differently sized PCR-products. In the new Ethopian species of *Diadegma*, a DNA fragment of approximately 780 bp was obtained, while in *D. insulare, D. mollipla, D. fenestrale* and *D. semiclausum* a slightly longer band of about 800 bp was visible on agarose gels. PCR-products of the ITS2 region of *D. leontiniae* and *D. rapi* had a length between 840 bp and 850 bp, respectively. Species-specific diagnostic patterns were generated with 11 different restriction enzymes but one enzyme (C*fo*I) distinguished all the tested species of *Diadegma* (Wagener et al., 2004b). The maximum interspecific nucleotide divergence between *D. mollipla* and *D. semiclausum* was 6.8% whereas the new *Diadegma* species exhibited about 35% divergence. The intraspecific divergence was 0.3% and 0.7% in *D. mollipla* and *D. semiclausum*, respectively (Wagener et al., 2004a).

Parasitoid strains may be associated with specific *P. xylostella* strains in a region suggesting that effective biocontrol agents should be obtained from the area of origin of a pest strain. For example, two Ethiopian species of *Diadegma* did not parasitize French populations of *P. xylostella* (Kirk et al., 2004). If the release of an exotic biocontrol agent is necessary and compatible with the target host strain then it is also important to evaluate its establishment and spread in the new habitat after its release.

1.8.5 Bottom-up Effects on Parasitoids

Plutella xylostella parasitism by *D. insulare* is affected by the quality of both the herbivore and its host plant (Chapter 5). For example, "high quality" *P. xylostella* larvae feeding on plants grown at high nitrogen levels yielded 93% female wasps while "low quality" *P. xylostella* developing on plants grown at low nitrogen levels yielded 58%

females (Fox et al., 1990). Different host plants can also have a significant impact on parasitism and performance of parasitoids (Chapters 6 and 7). Parasitism by *D. semiclausum* was greater on host larvae fed on common cabbage (*Brassica oleracea* (L.) var. *capitata*) than on larvae on cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*) or Chinese cabbage (*B. campestris* L. ssp. *pekinensis*), but parasitism by *C. plutellae* was greatest on larvae from Chinese cabbage compared with the other three host plants (Talekar and Yang, 1991). These researchers stated that they could not provide any plausible explanation for such differences in parasitism. However, subsequent research revealed that plant volatiles from Chinese cabbage were more attractive to *C. plutellae* than those of common cabbage resulting in 4- to 15-fold higher parasitism of *P. xylostella* larvae feeding on Chinese cabbage (Liu and Jiang, 2003). In a choice test, *P. xylostella* larvae feeding on sugar snap peas, *Pisum sativum* L. (Fabaceae) and collards, *B. oleracea* var. *acephala* were exposed to *D. mollipla*. The parasitism rate of *P. xylostella* larvae on sugar snap peas was four times higher than that on collards and the authors speculated that crucifer volatiles were unlikely to be used by this parasitoid for host location (Löhr and Rossbach, 2004). Prior to this study, *D. mollipla* was recorded as an important parasitoid of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), on potato and tobacco in South Africa (Broodryk, 1971) suggesting that *D. mollipla* is only loosely associated with the crucifers and *P. xylostella* (Löhr and Rossbach, 2004). Studies on other *Diadegma* species could generate more reliable results regarding any impact of such host-plant shift by *P. xylostella*.

Idris and Grafius (1996) assessed the impacts of various cultivated and wild crucifers on parasitism success of *D. insulare*. Highest *P. xylostella* parasitism was recorded on *Sinapis arvensis* L. (=*Brassica kaber)* (91.5%) followed by *B. oleracea* var. *italica* (90.8%), *B. oleracea* var. *botrytis* (89.5%), *B. napus* L. (87.3%), *B. oleracea* var. *acephata* (83.3%), *B. nigra* (L.) (81.3%), *B. oleracea* var. *capitata* (79.5%), *Raphanus raphanistrum* L. (72.8%), *Erysimum cheiranthoides* L. (65.5%), *Thlaspi arvense* L. (65.0%), *Berteroa incana* (L.) (55.0%) and *Lepidium campestre* (L.) (39.3%). No parasitism occurred on *Capsella bursa-pastoris* (L.) and no *P. xylostella* larvae survived on *Barbarea vulgaris* R. Br. Developmental time of parasitized third instars to pupation was about 13 days on most of the tested plants. Larval developmental time increased on *E. cheiranthoides* (15.8 days), *B. incana* (16.0 days), *T. arvense* (16.3 days) and *L. campestre* (20.3 days). However, these results should be interpreted with caution because the nutrient compositions of the leaves used in this test might be quite different from each other given they were collected from different locations on the Michigan State University campus.

1.9 Natural Enemies: Entomopathogens

Parasitoids are fundamental for an integrated and sustainable management program of *P. xylostella*, but supplemental control through the application of an insecticide is necessary when an economic threshold is exceeded (Shelton *et al.*, 1983). Because of their specificity against target pests and minimal environmental impacts, microbial pest control agents (MPCAs) are welcome additions to integrated pest management programs. The use of microbial insecticides in crop protection is increasing with the discovery and development of new entomopathogens. The potential of various entomopathogens including bacteria, fungi, viruses, protozoa, and nematodes as biocontrol agents has been tested for *P. xylostella* management but few studies have demonstrated practical promising significance (Fig. 1).

1.9.1 Bacteria

1.9.1.1 *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae)

The Gram-positive soil bacterium, *Bacillus thuringiensis (Bt)*, is a complex of subspecies characterized by production of parasporal crystalline inclusions during sporulation. These inclusions are comprised of relatively high quantities of one or more glycoproteins known as δ-endotoxins or insecticidal crystal proteins (ICP's) that play a vital role in *Bt* pathogenicity to insects and other invertebrates (Bauer, 1995). Since the launch of the first commercial *Bt*-product in France in 1938, over 100 *Bt*-based pesticides have been introduced across the world and currently δ-endotoxins are active ingredients in more than 90% of all MPCAs (Glare and O'Callaghan, 2000). *Bt*-based products are the most promising alternatives to conventional insecticides because they are highly toxic to certain pests and are compatible with integrated pest management strategies due to their narrow host specificity, high amenability to genetic engineering, and because they cause little or no harm to humans, most beneficial insects and other non-target organisms (Tabashnik, 1994; Bauer, 1995). In certain parts of the world *P. xylostella* has exhibited considerable resistance to *Bt*-based products (Sarfraz, 2004). In principle, any interference with the cascade of steps associated with the mode of action of *Bt* Cry proteins (solubilization, proteolytic activation, passage through the peritrophic membrane, receptor binding, membrane insertion, pore formation, and osmotic lysis of midgut cells) may help the insect to develop resistance (Tabashnik, 1994). Evidence exists that binding site alteration is a major mechanism involved in *P. xylostella* resistance to *Bt* (Ferré and van Rie, 2002; Sarfraz, 2004).

The first case of field resistance to *Bt* was reported from Hawaii in 1990 when *P. xylostella* field-collected populations displayed 30-fold resistance to Dipel® (*Btk*, *B. thuringiensis* subsp. *kurstaki*) (Tabashnik et al., 1990). Laboratory screening of this colony with Dipel® increased resistance rapidly and the resulting colony (NO-QA) was more than 6,800-fold resistant to Cry1Ac (Tabashnik et al., 1993). Tang et al. (1996) demonstrated that the Loxa-A colony from Florida was greater than 1,500-fold resistant to Javelin® (*Btk* NRD12) in the second generation. In the absence of selection, resistance rapidly declined to about 300-fold but remained stable at this level in subsequent generations. The insects with this stabilized level were more than 200-fold resistant to Cry1Aa, Cry1Ab, and Cry1Ac, but they were still fully susceptible to Cry1B and Cry1C. Resistance in *P. xylostella* populations to *Btk* products resulted in heavy use of *Bta (B. thuringiensis* subsp. *aizawai)* that typically contains Cry1C, in addition to Cry1A toxins. Two Hawaiian colonies (NO-93, NO-95) displayed up to 20-fold resistance to Cry1Ca and were found to be only 2- to 4-fold resistant to *Bta* and 50- to 130-fold less susceptible to *Btk* formulations when compared with a susceptible colony (Liu et al., 2000). A field-collected population (Cry1C-Sel) from South Carolina displayed 31-fold resistance to Cry1C. Continuous laboratory selection using first Cry1C protoxin, and in later generations transgenic broccoli expressing high levels of Cry1C, increased resistance to 12,400-fold (in neonates) and 63,100-fold (in second instars) after 26 generations (Zhao et al., 2000). A Malaysian colony (SERD3) was reported to be resistant to both *Btk* and *Bta*. After rearing in the laboratory for seven generations in the absence of any selection, *P. xylostella* showed 330-fold and 160-fold resistance to *Btk* and *Bta*, respectively. Furthermore, selection during the next three generations with *Bta* increased resistance to *Bta* but only marginally to *Btk* and vice versa (Wright et al., 1997; Ferré and van Rie, 2002).

Bt products are still effective against *P. xylostella* in many crucifer producing regions and need to be used judiciously to conserve their efficacy. Braun et al. (2004) collected *P. xylostella* from different sites in Saskatchewan, Canada in 1998 and 2001 and tested over 4600 larvae using commercial preparations of Dipel®. All the assayed larvae showed complete susceptibility to recommended field application rates.

1.9.2 Fungi

Bt and fungi are quite dissimilar in their modes of action. The ICPs and/or spores of the bacterium must be ingested by a larva to elicit an effect, while conidia of the fungus must contact insect cuticle, germinate, and penetrate it through enzymatic action and mechanical pressure. *Bt* kills usually within 36 to 48 h by paralyzing the midgut, damaging columnar epithelial cells, and initiating septicemia. Conidia of the fungus germinate, penetrate through the integument and initiate mycelial growth, resulting in death of insect in days (Glare and O`Callaghan, 2000; Inglis et al., 2001).

Several species of fungal pathogens including *Zoophthora radicans* (Brefeld) Batko, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metsch.) Sorokin, *Paecilomyces farinosus* (Holm ex Gray) Brown and Smith, *Fusarium* spp., *Pandora* spp., *Erynia* spp., *Conidiobolus* spp., and *Scopulariopsis* spp. have been isolated from *P. xylostella* (Vandenberg et al., 1998; Cherry et al., 2004a; Kirk et al., 2004) but few have been studied in detail. Vandenberg et al. (1998) reported screening 55 isolates of different fungi including *B. bassiana, M. anisopliae, P. farinosus* and *Fusarium* sp. for *P. xylostella*.

1.9.2.1 *Zoophthora radicans* (Brefeld) Batko (Zygomycetes: Entomophthorales)

Zoophthora (=*Erynia*) *radicans*, an important entomopathogenic fungus isolated from *P. xylostella*, is reported to cause epizootics under favorable environmental conditions and can reduce local populations to zero (Ooi, 1981; Riethmacher et al., 1992). The moths infected/contaminated with *Z. radicans* can serve as a source of fungal inoculum in the field and airborne conidia lead to epizootics as a result of auto-dissemination in *P. xylostella* populations (Vickers et al., 2004). Although infected insects can survive for some period of time, a reduction in feeding damage may occur sooner. For example, third-instar infected larvae consumed 44% less foliage than healthy larvae (Furlong et al., 1997). Moths infected with this fungus laid significantly fewer eggs (Furlong et al., 1997) and *Z. radicans* was also found to disrupt the mating behavior of *P. xylostella* by inhibiting the response to and production of sex pheromone (Reddy et al., 1998).

1.9.2.2 *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes: Moniliaceae)

There is increasing interest in the use of mycoinsecticides based on *Beauveria bassiana* for the control of *P. xylostella*. This pathogen applied at the rate of $3\text{x}10^6$ conidia ml^{-1} gives 100% *P. xylostella* mortality after 3 to 7 days (Furlong, 2004) and contaminated moths effectively transmit (horizontal/passive transmission) this fungus to healthy moths and larvae foraging on plants. Sporulating cadavers producing *Z. radicans* or *B. bassiana* conidia result in similar transmission rates (Furlong and Pell, 2001). Various commercial formulations of this fungus significantly suppressed the *P. xylostella* populations in screened enclosures and the field (Shelton et al., 1998; Vandenberg et al., 1998; Becker, 1999).

1.9.2.3 Other Fungi

Jun (2000) tested infectivity of five isolates of *M. anisopliae* and one isolate of *Nomuraea rileyi* (Farlow) Sampson for *P. xylostella*. One isolate of *M. anisopliae* was highly infective with an LC_{50} of 2.03 x 10^4 conidia ml^{-1} and an LT_{50} of 4.97 days at 10^7 conidia ml^{-1}. The *N. rileyi* gave high mortality but did not sporulate in any of the cadavers (Jun, 2000). *Metarhizium anisopliae* isolated from *P. xylostella* collected in eastern Romania was also very virulent (Kirk et al., 2004).

1.9.3 Viruses

A number of baculoviruses infect *P. xylostella*. In a review of the potential viruses, Wilding (1986) concluded that granuloviruses (GVs) showed promising levels of pathogenicity. A Kenyan isolate of PxGV (Nya-01) applied in the field at 3.0 x 10^{13} occlusion bodies (OB) ha^{-1} controlled *P. xylostella* on kale with 82% and 90% infection rates for second and first instars, respectively (Grzywacz et al., 2004). Second instars of the Benin *P. xylostella* strain were >90-fold more susceptible to this Nya-01 isolate than the Kenyan strain, and seedlings emerging from PxGV-loaded soils acquired adequate virus to infect *P. xylostella* larvae feeding on them (Cherry et al., 2004b). The PxGV-fed larvae consumed less foliage and the proportion of decreasing total leaf consumption from the first through fourth instars was 93%, 77%, 53%, and 46%, respectively compared with their healthy counterparts (Lü et al., 2004). The PxGVs have been reported from various countries including Japan (Asayama and Osaki, 1970), Taiwan (Kadir, 1986), India (Rabindra et al., 1997), China (Kadir et al., 1999a) and Kenya (Grzywacz et al., 2004). Five Kenyan isolates of PxGV were found to be a mixture of different genotypes on the basis of sub-molar bands in their REN (restriction endonuclease) fragment profiles and their LD_{50} values for *P. xylostella* neonates were not significantly different from the Chinese, Japanese and Taiwanese strains (Woodward et al., 2004).

In China, a nucleopolyhedrosis virus isolated from *P. xylostella* (PxMNPV) was characterized as genetically distinct from AcMNPV and AfMNPV. Based on relative differences in LC_{50}s, PxMNPV was three to four log cycles more potent against *P. xylostella* than either AcMNPV or AfMNPV (Kariuki and McIntosh, 1999). The NPVs isolated from *Anagrapha falcifera* (Kirby) (AfMNPV), *Autographa californica* (Speyer) (AcMNPV), and *Galleria melonella* (L.) (GmMNPV) were also infectious to *P. xylostella* (Kadir *et al.*, 1999b) but their potency was moderate to low, even after serial passage in this insect (Farrar and Ridway, 1999).

A cypovirus (PxCPV) was isolated from a single *P. xylostella* larva in 1999 in an experimental field in Greater Accra region of Ghana. Like other CPVs, its replication was restricted to the midgut but it had very low virulence with an estimated LC_{95} value of greater than 9.0×10^{10} OB ml^{-1} for third-instar *P. xylostella* larvae (Cherry et al., 2004a).

1.9.4 Other Entomopathogens

Nematodes and microsporidia have also been reported to cause infection to *P. xylostella* (Baur et al., 1997, 1998; Haque et al., 1999; Idris et al., 2002, 2004) but they need more extensive laboratory and field evaluations aimed at optimizing effectiveness. A nematode, *Steinernema carpocapsae* (Weiser), tested against *P. xylostella* on *Nasturium aquaticum* (L.) Hayek farms in Hawaii gave 41% control of the pest alone and 58% when it was applied in conjunction with *Btk* (Baur et al., 1998).

A microsporidian, *Vairimorpha* sp., isolated from *P. xylostella* collected from Sundai Palas, Cameron Highlands (Malaysia) caused 100% mortality at 1.5 x 10^3 spores per larva. The time to achieve 90 to 100% mortality was dose-dependent and varied from five days with 1.5 x 10^6 spores per larva to 11 days at 1.5 x 10^3 spores per larva (Haque et al., 1999). *Plutella xylostella* larval infection by *Nosema bombycis* Negali was significantly higher in the Cameron Highlands (71.3%) than in the Serdang-Gombak lowlands (10.0%). Mortality was higher in younger instars (I and II) than older instars even at lower concentration of 4,260 spores μl^{-1} (Idris et al., 2004). Parasitoids such as *D. semiclausum* were involved in horizontal transmission of microsporidia spores among *P. xylostella* larvae (Idris et al., 2004).

1.10 Interactions Between Parasitoids and Entomopathogens

When an insect pest is exposed to more than one killing agent, there is the possibility of an interaction that can enhance, limit or both limit and enhance various aspects of the effectiveness of control measures (Furlong and Pell, 1996, 2000). Further, as the parasitoids actively forage for host larvae on crops, they can come into close contact with pathogens applied for the control of pest. It is, therefore, also important to investigate interactions which may occur between parasitoids and entomopathogens.

Bt toxins did not have detrimental impacts on adult *C. plutellae* (Chilcutt and Tabashnik, 1999) and the braconid successfully completed its larval development in *Bt*-resistant *P. xylostella* larvae feeding on *Bt* transgenic crucifers. *Bt*-resistant (BTR) *P. xylostella* larvae caused significantly greater damage to *Bt* leaves than *Bt*-susceptible (BTS) larvae and BTR-damaged leaves were more attractive to adult *C. plutellae* females than those damaged by BTS larvae. Of the 40 parasitoids tested, 27 landed on BTR-damaged leaves and only seven chose BTS-damaged leaves; six wasps did not respond. Parasitoid females did not distinguish between *Bt* and non *Bt* plants both damaged by BTR larvae. For example, BTR-damaged *Bt* and non *Bt* leaves were offered to 40 wasps; 19 flew to *Bt* leaves and 17 to non *Bt* while four did not show any response. These findings suggest that *C. plutellae* is equally effective in controlling *Bt*-resistant *P. xylostella* on genetically modified *Bt* plants and non *Bt* plants (Schuler et al., 2003). *Bt*-derived commercial insecticides (Xentari® and Crymax®) were found safe for *D. insulare* adults and pupae (Xu et al., 2004).

Furlong and Pell (1996) observed that *Z. radicans* did not infect *C. plutellae*. However, its infection of *P. xylostella* larvae before and four days after parasitism by *D. semiclausum* killed both host and the parasitoid (Furlong and Pell, 2000). *Diadegma semiclausum* showed 100 times less susceptibility to *Z. radicans* than the *P. xylostella* and has never been found infected in the field during epizootics (Pell et al., 2001). Increased concentrations (greater than 1×10^6 conidia ml^{-1}) of *B. bassiana* showed significant adverse effects on *D. semiclausum* cocoon production and adult wasp emergence; parasitoid larvae within the host also became infected by the fungus (Furlong, 2004). Microsporidia may affect parasitoids in different ways. For example, progeny of *Trichogramma chilonis* Ishii severely declined when they parasitized eggs of *P. xylostella* infected with *Vairimorpha* species. The longevity and reproductive performance of parasitoids that emerged from infected hosts also reduced significantly (Schuld et al., 1999). *Nosema bombycis* infection altered diurnal activities of adult *D. semiclausum* and infected females spent significantly more time on activities that were not related to parasitism, for example, more moving (65%) than resting (25%), flying (5%), feeding (4%) and grooming (1%) (Idris et al., 2004).

van Emden (1991) argued that insecticide concentrations could be reduced three fold on partially resistant host plants without appreciable increases in the pest population. Reducing insecticide usage in an insect pest management program can also be achieved through integration with biological control agents. Biocontrol of *P. xylostella* is an important measure that can be integrated into insect management programs, particularly when the pest complex is simple and *P. xylostella* is the dominant species (Hamilton et al., 2004).

Crucifer growers should choose cultivars that are partially resistant to *P. xylostella* and more attractive to its natural enemies. They should avoid unnecessary use of fertilizers as it may result in higher infestations of crucifer pests such as *Delia* species (Dosdall et al., 2004a). Based on pest scouting and monitoring, a selective insecticide (e.g. bacterial and/or fungal products) can be used to keep the pest population to a single action threshold level (i.e. minimum acceptable pest infestation level) available to parasitoids. *Bt*-transgenic crops grown in conjunction with refugia may also enhance sustainable pest management. Refuge plants can serve as reservoirs for both the pest and its natural enemies. In theory, the majority of the insects infesting *Bt*-plants will be killed by *Bt*-toxins and the survivors (*Bt*-resistant) may serve as hosts for parasitoids that will be present in refugia.

The design of an integrated approach requires that the characteristics of each control agent be documented carefully. Only with this knowledge can the most effective strategy be employed. For example, *C. plutellae* is less efficient at host searching, prone to multiparasitism, and *P. xylostella* larvae parasitized by this braconid live for longer periods resulting in more foliage consumption than in unparasitized counterparts. Current data indicate an approach that whenever possible, larval parasitoids in particular a *Diadegma* species and pupal parasitoids such as *Diadromus* species should be introduced, keeping in mind the native parasitoid complex in a given region. Application of *Bt*-toxins or other selective insecticides should be made to suppress the pest population below the single action threshold level. Remaining individuals will provide hosts for the larval parasitoids, and successfully pupating insects will serve as hosts for pupal parasitoids.

Growers need both the information and tools to manage *P. xylostella* and other pests. The co-ordinated use of biocontrol agents can lead to effective pest control. Overall this strategy will help maintain the viability of crucifers in many regions especially where resistance to insecticides is common.

Table 1-I. Examples of cultivated brassicaceous host plants of *Plutella xylostella*.

Species/Cultivar	Common Name(s)	Selected Reference(s)
Brassica napus L.	canola, Canadian turnip, rutabaga	Idris and Grafius, 1996; Brown et al., 1999
Brassica rapa L. (=*B. campestris* (L.))	turnip rape, turnip green, field mustard	Brown et al., 1999; Ulmer et al., 2002
Brassica rapa L. var. *pakchoi*	pak choi	Talekar and Shelton, 1993
Brassica rapa L. var. *pekinensis*	Chinese cabbage	Talekar et al., 1994; Liu and Jiang, 2003
Brassica carinata L.	Ethiopian mustard	Ayalew et al., 2004
Brassica juncea (L.)	Indian mustard, brown mustard	Bodnaryk, 1997; Brown et al., 1999
Brassica napa L.	turnip	Abro et al., 1994
Brassica nigra (L.)	black mustard	Idris and Grafius, 1996
Brassica oleracea L. var. *acephala*	collard, flowering kale	Idris and Grafius, 1996
Brassica oleracea L. var. *alboglabra*	kale	Talekar and Shelton, 1993
Brassica oleracea L. var. *botrytis*	cauliflower	Idris and Grafius, 1996; Reddy et al., 2004
Brassica oleracea L. var. *capitata*	cabbage	Abro et al., 1994; Idris and Grafius, 1996
Brassica oleracea L. var. *gemmifera*	Brussels sprouts	Talekar and Shelton, 1993
Brassica oleracea L. var. *gongylodes*	kohlrabi	Reddy et al., 2004
Brassica oleracea L. var. *italica*	broccoli	Idris and Grafius, 1996; Reddy et al., 2004
Raphanus sativus L.	radish, bier radish	Abro et al., 1994
Sinapis alba L. (= *Brassica hirta* Moench)	white mustard, yellow mustard	Bodnaryk, 1997; Brown et al., 1999

Table 1-II. Examples of wild brassicaceous host plants of *Plutella xylostella*.

Species	**Common Name (s)**	**Selected Reference(s)**
Arabidopsis thaliana (L.) Heynh	thalecress, mouse-earcress	Ratzka et al., 2002; Barker et al., 2004
Barbarea vulgaris (L.) R. Br.	yellow rocket, rocketcress	Idris and Grafius, 1996; Shelton and Nault, 2004
Berteroa incana L. DC	hoary alyssum	Idris and Grafius, 1996
Capsella bursa-pastoris (L.)	shepherd's purse, mother's-heart	Idris and Grafius, 1996
Cardamine flexuosa With.	flexuous bittercress	Muhamad et al., 1994;
Descurainia sophia (L.)	flixweed	Talekar and Shelton, 1993
Erysimum cheiranthoides L.	wormseed mustard, treacle mustard	Renwick and Radke, 1990; Idris and Grafius, 1996
Lepidium campestre (L.) R. Br.	field pepperweed	Idris and Grafius, 1996
Lepidium virginicum L.	Virginia pepperweed, peppergrass	Muhamad et al., 1994; Begum et al., 1996
Raphanus raphanistrum L.	wild radish, wild rape, wild turnip	Idris and Grafius, 1996
Rorippa indica (L.) Hiern	Indian marshcress	Muhamad et al., 1994; Begum et al., 1996
Rorippa islandica (Oeder) Barbàs	marsh yellowcress	Muhamad et al., 1994
Sinapis arvensis L. (= *Brassica kaber* (DC) Wheeler)	wild mustard, crunchweed	Idris and Grafius, 1996
Sisymbrium altissimum L.	tumbling mustard, tall hedge mustard	Talekar and Shelton, 1993
Thlaspi arvense L.	stinkweed, pennycress, Frenchweed	Idris and Grafius, 1996

Table 1-III. Examples of non-brassicaceous plants on which *Plutella xylostella* is known to survive/develop.

Species	Family	Common Name	Reference(s)
Tropaeolum majus L.	Tropaeolaceae	nasturtium	Renwick and Radke, 1990
Cleome species	Capparidaceae	spider plant	Sarfraz et al., 2005b
Pisum sativum L.	Fabaceae	peas	Gupta and Thorsteinson, 1960a; Löhr, 2001; Löhr and Gathu, 2002
Hibiscus esculentis L.	Malvaceae	okra	Gupta, 1971

Table 1-IV. Biodiversity and developmental parameters associated with major parasitoids of *Plutella xylostella*.

Parameters	*Diadegma* species	*Microplitis* species	*Cotesia* species	*Oomyzus* species	*Diadromus* species	References
Number of species commonly associated with *P. xylostella*	9	2	1	1	2	Hardy, 1938; Talekar and Shelton, 1993; Azidah et al., 2000; Kirk et al., 2004
Host stage attacked	Larval	Larval	Larval	Larval-Pupal	Prepupal-Pupal	Lloyd, 1940; Xu et al., 2001a; Shi et al., 2004
Life style	Solitary	Solitary	Solitary	Gregarious	Solitary	Lloyd, 1940; Putnam, 1968; Ooi, 1988; Rowell, 2004
Adult body size (mm)	5 to 7	2.3 to 2.5	2.5 to 2.6	1.3 to 1.4	6 to 7	Gharuka et al., 2004; Rowell, 2004
Evidence for pesticide resistance	Yes	?	Yes	?	?	Xu et al., 2001b; Liu et al., 2004; Rowell, 2004
Number of larval instars	4	3	4(?)	?	?	Bolter and Laing, 1983; Shi et al., 2002; Gharuka et al., 2004
Average developmental time (egg to adult) (days)	12 to 18	12 to 18	13 to 18	13 to 17	15 to 17	Bolter and Laing, 1983; Ferreira and Torres, 2003; Talekar, 2004; Rowell, 2004
Optimum developmental temperature (°C)	20 to 22	20 to 22	26 to 28	26 to 28	22 to 26	Bolter and Laing, 1983; Liu et al., 2001; Talekar, 2004
Average thermal requirement	282 $^{\circ}D_{6.6}$ (*D. insulare*)	218 $^{\circ}D_{9.2}$ (*M. plutellae*)	?	211.8 $^{\circ}D_{11.6}$ (*O. sokolowskii*)	225.1 $^{\circ}D_{7.4}$ (*D. collaris*)	Bolter and Laing, 1983; Liu et al., 2001; Ferreira and Torres, 2003

Table 1-V. *Diadegma* species known to parasitize *Plutella xylostella* throughout the world.

Diadegma species	Distribution	Reference(s)
D. insulare (Cresson) (= *D. plutellae, D. hellulae, D. congregrator, D. polynesialis, D. pygmaeus)*	Nearctic to the northern Neotropical region and some Pacific islands, including Hawaii (only one species is known to attack *P. xylostella* in North America)	Fitton and Walker, 1992; Azidah et al., 2000; Kirk et al., 2004; Wagener et al., 2004b
D. fenestrale (Holmgren) (= *D. varuna, D. niponica, D. gracilis*)	Palaearctic and south-east to Sri Lanka and the Philippines	Fitton and Walker, 1992; Azidah et al., 2000; Wagener et al., 2004b
D. leontiniae (Bréthes)	Southern Neotropical region: Argentina, Uruguay	Fitton and Walker, 1992; Azidah et al., 2000; Kirk et al., 2004; Wagener et al., 2004b
D. mollipla (Holmgren)	Afrotropical, including some Indian Ocean and South Atlantic islands	Azidah et al., 2000; Kirk et al., 2004; Wagener et al., 2004b
D. exareolator Aubert	Great Britain	Shaw and Hortsmann, 1997
Diadegma sp. n.	Ehiopia	Wagener et al., 2004b; Kirk et al., 2004
D. novaezealandiae Azidah, Fitton & Quicke	New Zealand	Azidah et al., 2000
D. rapi (Cameron)	South-eastern Australia	Fitton and Walker, 1992; Azidah et al., 2000; Wagener et al., 2004b
D. semiclausum (Hellén) (= *D. xylostellae, D. eucerophaga, Angitia cerophaga*)	Palearctic, and many other parts of the world	Fitton and Walker, 1992; Azidah et al., 2000; Kirk et al., 2004; Wagener et al., 2004b

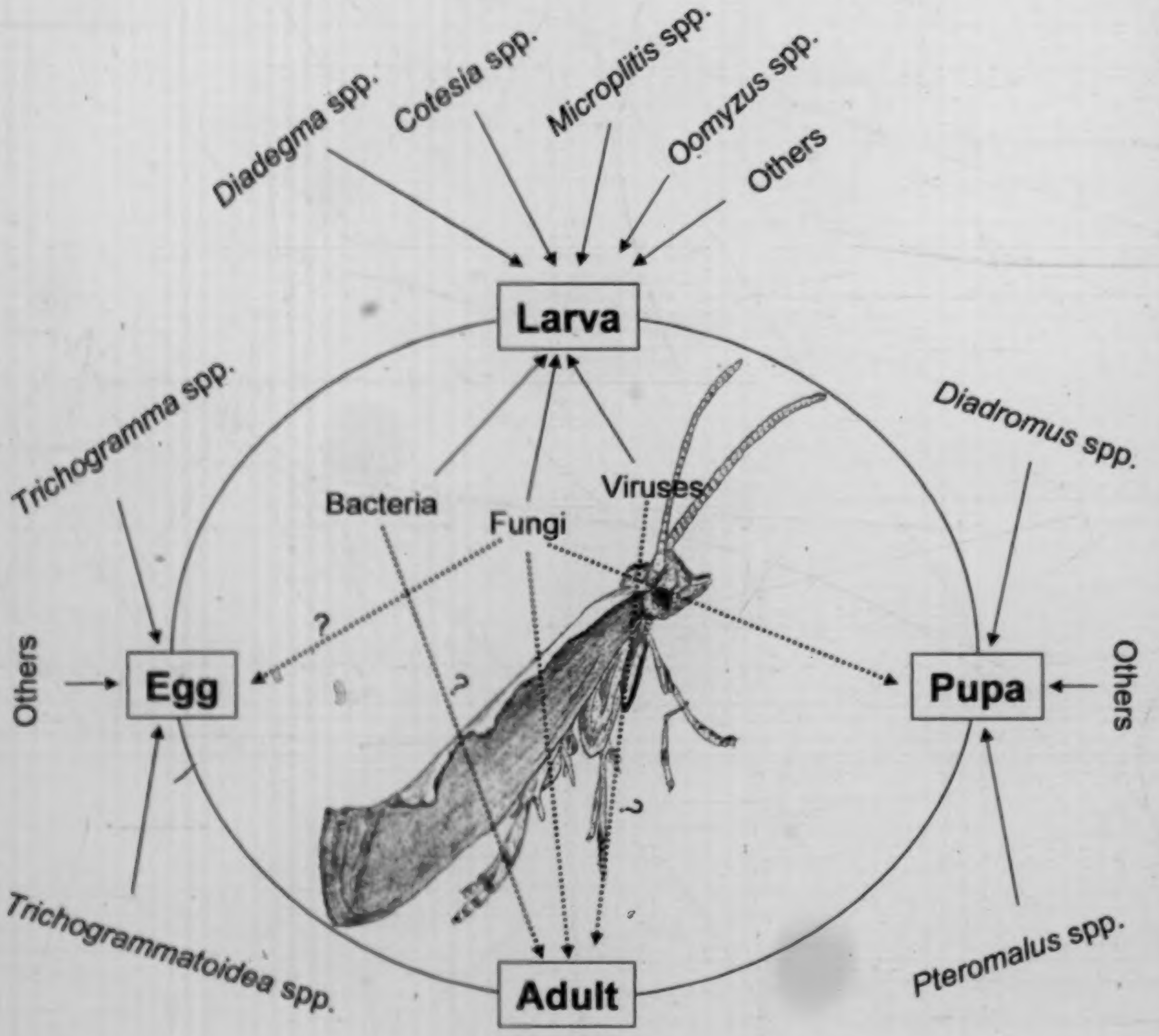

Figure1-I. Principal biocontrol agents of *Plutella xylostella*.

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Chapter 2:

Bottom-up Effects of Host Plant Nutritional Quality on *Plutella xylostella* and Top-down Effects of Herbivore Attack on Plant Compensatory Ability

2.1 Introduction

Understanding ecological interactions among insect herbivores and their host plants has long been a goal of ecologists. A substantial body of literature implicates host plant quality as a primary player influencing herbivore-host plant interactions (Thompson, 1988a; Louda and Collinge, 1992; Price, 1997). Here, the basic premise is that insects will prefer to oviposit and feed on plants that confer the greatest fitness to themselves and their offspring (Thompson, 1988b; Craig et al., 1989; Denno et al., 1990; Fox and Lalonde, 1993; Barker and Maczka, 1996; Craig and Ohgushi, 2002), and that natural selection will lead to a tight linkage between host plant preference and herbivore performance, especially for immatures that are reliant on their mothers for host selection (Craig et al., 1989; Renwick and Chew, 1994; Larsson and Ekbom, 1995).

The preference/performance hypothesis is based on the premise that plants differ in their quality as hosts for herbivorous insects. Numerous studies show that differences in plant quality reflect the availability of soil nutrients. Total numbers of leaves, main stem diameters and root masses are considered important plant morphology indices (Gartner, 1994; Tekeli and Ates, 2003). Variation in soil nutrients affects the production of plant defensive chemicals (Inbar et al., 2001; Marazzi et al., 2004; Ramona et al., 2005), and plant morphology and quality (Chau and Heinz, 2006), and in turn can influence the preference and performance of insect herbivores. Hypotheses of 'plant stress' and 'plant vigor' have been proposed to predict the responses of herbivores to soil nutrients, as mediated by host plant quality.

The plant stress hypothesis of White (1984) predicts that stressed plants serve as better hosts for insect herbivores. According to this hypothesis, plants under stress are more suitable for insect herbivores due to increased nutritional quality arising from reduced protein synthesis and increased free amino acids in plant tissues (White, 1969; 1984; Mattson and Haack, 1987), and reduced synthesis of defensive chemicals (Rhoades, 1979). The effects of host plant stress were often dependent on the insect feeding guild, the host plant species, and the nature and level of stress (Heinrichs, 1988; Larsson, 1989; Waring and Cobb, 1992). Certain insects (e.g. phloem feeders) prefer stressed plants whereas others (e.g. leaf chewers, leafminers) are negatively affected when feeding on stressed plants (Larsson, 1989; Koricheva et al., 1998; Inbar et al., 2001). For instance, whiteflies, *Bemisia tabaci* (Gennadius) and *B. argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), prefer stressed cotton plants (Flint et al., 1996; Skinner, 1996) while performance of grass miners, *Chromatomyia milii* (Kaltenbach) (Diptera: Agromyzidae), significantly declines on stressed host plants (Scheirs and Bruyn, 2005).

In contrast, the plant vigor hypothesis of Price (1991) proposes that herbivorous insects will prefer and perform better on fast-growing, vigorous plants or plant modules compared with slow-growing, less vigorous plants or plant modules. The hypothesis refers particularly to those insects that have a tight connection between adult oviposition site selection and larval feeding guild, and insects whose larval development is closely linked with host plant growth processes. For instance, the majority of galling and mining insects that have the most intimate relationships with their host plants seem to be the most suitable candidates to support this hypothesis (Price, 1991). However, the underlying mechanisms of the plant vigor hypothesis are not known.

Empirical evidence exists in support of both hypotheses. Some studies have shown that insects perform better on stressed plants (White, 1969, 1984; Mattson, 1980; Jones and Coleman, 1988), but others indicate that vigorous plants frequently support higher densities of insect herbivores than their stressed counterparts (Leigh et al., 1970; Fox et al., 1990; Price, 1991; Meyer and Root, 1996; Inbar et al., 2001; Craig and Ohgushi, 2002; Chen et al., 2004; Dosdall et al., 2004a; Heisswolf et al., 2005). There is also evidence that some insect herbivores do not discriminate between stressed and vigorous host plants. For example, *Diplolepis ignota* Osten Sacken, *D. nodulosa* Beutenmüller, and *D. rosaefolii* Cockerell (Hymenoptera: Cynipidae) respond similarly to vigorous and stressed rose plants (Williams and Cronin, 2004). Host plant nutrional quality can therefore ameliorate, exacerbate, or have no effect at all on the host preference and performance of insect herbivores.

In the current study, I examined the effect of different nutrient regimes viz. no added soil fertility (stressed plants), two levels of intermediate soil fertility (average plants) and two levels of high soil fertility (vigorous plants) on various growing parameters of *Brassica napus* L. (Brassicaceae). These plants were used as hosts for the oligophagous herbivore *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), for which several fitness correlates were measured. Data were initially used to test for a relationship between plant preference and insect performance to better understand the ecological interactions and mechanisms involved in host plant selection and utilization by insect herbivores. Data were used subsequently to test predictions of the plant stress hypothesis versus the plant vigor hypothesis.

2.2 Materials and Methods

2.2.1 Insects and Plants

The laboratory colony of *P. xylostella* was maintained in the greenhouse and growth chambers on potted *B. napus* cv. Q2 plants at 22±0.5°C with 16h L: 8h D. Moths collected from different commercial fields throughout Alberta, Canada were added to the culture every summer to maintain genetic diversity. *Plutella xylostella* normally oviposit on the adaxial leaf surfaces with a range of 11 to 188 eggs per female (Harcourt, 1957; Talekar and Shelton, 1993). Neonates are usually leafminers that feed on the spongy mesophyll tissue while instars 2 to 4 are leaf mass consumers that normally feed on the abaxial leaf surfaces; pupation takes place on host plants or any other suitable substrate (Harcourt, 1957).

Brassica napus plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium. Treatment plants were grown under four fertility regimes, viz. 0.5, 1.0, 3.0, 5.0 g pot^{-1} of 20: 20: 20 (nitrogen: phosphorous: potassium) (Plant Products Co. Ltd., Brampton, Ontario, Canada), while control plants were grown without any added fertilizer. Fertilizer treatments were applied in two split applications to avoid any phytotoxic effects of higher concentrations: the first application was made after the second week of seed germination while the second application was made when plants were three weeks old. Each fertilizer treatment was applied after dissolving concentrate in 100 ml tap water while control plants received 100 ml water only. Four-week-old plants were used for all experiments.

2.2.2 *Plutella xylostella* Oviposition: Free-Choice Experiments

2.2.2.1 Intact Plant Study

Ovipositional preference experiments were conducted with *P. xylostella* in a multiple choice manner in ten screened cages (120 x 120 x 120 cm) under greenhouse conditions using a randomized complete block design and each cage was considered a block. The entire experiment was repeated i.e., a total of 20 cages were used (see section 2.2.5 for analysis). One plant from each fertilizer-treatment and the water-only control were placed randomly into each cage (i.e., five plants per cage). A total of 20 adults (four moths per plant) were released in each cage supplemented with a 10% honey solution for adult feeding. Moths were always ≤1 d old and were released in 1:1 (m: f) sex ratio from a container placed in the centre of the cage. Eggs on each plant were counted 3 d after releasing the moths.

2.2.2.2 Leaf Disc Study

It was suspected that leaf areas and/or leaf numbers might have influenced the *P. xylostella* oviposition in the above intact plant study (section 2.2.2.1). In this experiment, therefore leaf discs of equal area (diameter = 4.0 cm) were taken from each treatment and control plant and were placed on moist filter papers in 15-cm Petri plates; a total of 10 plates were used and each plate was considered a block. Leaf discs were placed equidistantly from the center of the Petri plate. A one-day-old mated female was released in the center of each plate, and the numbers of eggs deposited on each leaf disc were recorded after 24 h. This experiment was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D).

2.2.3 Effects of Different Fertilizer Treatments on Insect Life History Traits

2.2.3.1 Intact Plant Study: Survival of *P. xylostella*

Over 100 newly emerged *P. xylostella* were allowed to oviposit on tinfoil sheets treated with an extract of *B. napus* leaves (Shelton et al., 1991). After 24 h, egg sheets were collected, excised in pieces each containing about 15 to 20 eggs, and incubated in individual plastic cups. Pre-imaginal survival was assessed in screened cages (40 x 40 x 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant, grown while subjected to one of the four fertilizer regimes or without added fertilizer (five treatments); the entire experiment used 10 plants per treatment and 50 cages. Five plants from each treatment were infested with first-instar larvae while the remainder served as uninfested controls. The first-instar larvae were gently shaken from incubation cups allowing them to spin silk threads, and 10 randomly selected neonates were then transferred onto each plant by holding them from the silk thread with a brush. The larvae on each plant were observed daily for pre-imaginal development and the number of surviving individuals was recorded. Pupae were harvested, weighed and kept individually in transparent plastic cups until adult emergence.

2.2.3.2 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

This experiment was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D). Excised leaves were placed on moist filter papers (9-cm diameter) in ventilated plastic containers. For each treatment, 100 larvae (eclosed as second instars within the preceding 24 h) were introduced into individual plastic containers; a total of 500 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Developmental times from second-instar larva to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were also weighed using a Sartorius Supermicro scale (Sartorius Inc., Edgewood, NY, USA). Adults were sexed; twenty pairs were used in experiments to determine longevity (without food), body weight and forewing area.

To quantify levels of larval feeding, all leaves damaged by *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA) and the final version (250 dpi) was saved as a TIFF file without LZW compression. Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory.

Twenty females and 20 males reared from each fertility regime were used to determine their longevities without food and their forewing areas. Moths were weighed within 24 h of their death. Their forewings were carefully removed, glued onto a paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX

Technologies Inc., Dallas, TX, USA), and wing areas were measured using Image J (Sarfraz et al., 2007).

2.2.4 Plant Parameters

2.2.4.1 Stem Diameter and Numbers of Leaves

To assess plant quality, stem diameter was measured at two points at the end of larval development experiment; one measurement was made at the root/shoot junction and another at 10 cm up the stalk from root/shoot junction using vernier calipers (Digital Calipers, Samona International). Total numbers of leaves per plant were also counted at the beginning of the experiment.

2.2.4.2 Tissue Nutrient Analysis

Five leaves from each replicate infested and uninfested (control) plant were collected at the end of larval development experiment, air-dried at room temperature, ground and subjected to nutrient analysis. The AOAC-990.03 reference method was followed for determination of total nitrogen and sulfur (AOAC, 2003a) while calcium, phosphorous, potassium, magnesium and sodium were assessed by using AOAC-985.01 in Norwest Laboratories, Lethbridge, Canada (AOAC, 2003b).

2.2.4.3 Root Masses: Plant Response to Herbivory

At the end of the life history traits experiment, plants were uprooted; their roots were carefully washed as described by Gartner (1994) and air-dried under room temperature. The roots were removed and weighed to determine the effects of different soil fertility regimes on root development when infested and not infested by *P. xylostella* larvae.

2.2.5 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used throughout as necessary to achieve normality and homoscedasticity before analyses (Steel et al., 1997), but untransformed means are presented. Analysis of variance (ANOVA) (PROC GLM) for a randomized complete block design was performed for oviposition choice experiments with cage/Petri plate as a blocking factor. ANOVA for a completely randomized design was performed as necessary to test the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). The data on leaf tissue nutrients and fitness correlates of female *P. xylostella* were also subjected to ordinations using PC-ORD4 software (McCune and Mefford, 1999). Correlations (PROC CORR) were determined between pupal weight and silk weight, pupal weight and adult weight, pupal weight and forewing area, pupal weight and adult longevity without food, adult weight and forewing area, and adult weight and longevity without food. T-tests (PROC TTEST) were performed for pair-wise comparison between female and male specimens for their development time, larval herbivory, pupal weight, silk weight, adult body weight, forewing area and longevity (without food) when they were reared on host plants grown under various soil-fertility regimes. PROC TTEST was also performed for pair-wise comparisons between infested and uninfested plants for various nutrient contents, and root masses of infested and uninfested (control) plants for each treatment separately.

Since the oviposition choice study was repeated, each repetition was considered as an independent experiment and tested if this experiment was repeatable or not. A new variable was generated (Experiment 1 and Experiment 2) and PROC GLM was performed to determine its level of significance in the ANOVA table, but combined data across both experiments are presented in Table 2-I.

2.3 Results

2.3.1 *Plutella xylostella* Oviposition: Free-Choice Experiments

2.3.1.1 Intact Plant Study

Fertilizer treatment had a highly significant effect on ovipositional preference by *P. xylostella* ($F_{4,36}$ = 84.32, $P < 0.0001$) (Table 2-I). Mean numbers of *P. xylostella* eggs deposited on plants fertilized at 1.0 g pot^{-1} significantly exceeded those deposited on untreated controls and plants treated with fertilizer at 3.0 and 5.0 g pot^{-1} (Table 2-I). Mean eggs deposited on plants subjected to fertilizer treatments of 5.0 g pot^{-1} were similar to those deposited on the untreated controls, but significantly lower than on plants treated with 0.5 and 3.0 g pot^{-1} (Table 2-I).

2.3.1.2 Leaf Disc Study

Fertilizer treatment significantly affected the ovipositional preference by *P. xylostella* on leaf discs ($F_{4,36}$ = 38.02, $P < 0.0001$) (Table 2-I). Overall oviposition preference was similar to that observed in intact plant study with most eggs deposited on leaf discs that were taken from plants subjected to fertilizer treatments of 1.0 g pot^{-1} (Table 2-I).

2.3.2 Effects of Different Fertilizer Treatments on Insect Life History Traits

Plutella xylostella survival varied significantly from neonate to pupa ($F_{4,16}$ = 14.50, $P < 0.0001$) and from pupa to adult ($F_{4,16}$ = 9.59, $P = 0.0004$) on plants grown under different fertility regimes (Table 2-II). Highest survival from neonate to pupa occurred on plants treated with 1.0 g pot^{-1} whereas least occurred on plants treated with 5.0 g pot^{-1}. Survival from neonate to pupa did not differ on plants subjected to treatments of 0.5, 1.0 and 3.0 g pot^{-1}. Lowest pupa to adult survival was observed on plants treated with 5.0 g pot^{-1} (Table 2-II).

Fertilizer applications strongly affected developmental time from neonate to pre-pupa, pupa to adult and neonate to adult for both females ($F_{4,76}$ = 10.18, $P < 0.0001$; $F_{4,76}$ = 9.63, $P < 0.0001$ and $F_{4,76}$ = 20.05, $P < 0.0001$ respectively) and males ($F_{4,76}$ = 15.35, $P < 0.0001$; $F_{4,76}$ = 4.95, $P = 0.0013$ and $F_{4,76}$ = 10.95, $P < 0.0001$ respectively) (Table 2-III). Pre-pupal development time was significantly affected for females ($F_{4,76}$ = 3.70, $P = 0.0083$), but not for males ($F_{4,76}$ = 1.74, $P = 0.1490$) (Table 2-III). Foliage consumption by female and male *P. xylostella* larvae varied significantly ($F_{4,76}$ = 12.47, $P < 0.0001$ and $F_{4,76}$ = 3.83, $P = 0.0069$ respectively) (Table 2-IV). Pupal weight and silk weight differed significantly for females ($F_{4,76}$ = 6.67, $P = 0.0001$ and $F_{4,76}$ = 4.23, $P = 0.0038$ respectively) and males ($F_{4,76}$ = 5.04, $P = 0.0012$ and $F_{4,76}$ = 14.68, $P < 0.0001$ respectively) when reared on host plants grown under various soil-fertility regimes (Table 2-IV). Fertilizer treatments significantly affected adult body weight, forewing area and longevity (without adult food) of females ($F_{4,76}$ = 4.55, $P = 0.0024$; $F_{4,76}$ = 5.58, $P = 0.0005$ and $F_{4,76}$ = 6.23, $P = 0.0021$ respectively) and males ($F_{4,76}$ = 9.98, $P < 0.0001$; $F_{4,76}$ = 11.06, $P < 0.0001$ and $F_{4,76}$ = 25.95, $P < 0.0001$ respectively) (Table 2-V).

Both female and male larval development was fastest on plants treated with 1.0 g fertilizer pot^{-1}, and significantly differed from those reared on plants treated with 0.0 and 5.0 g pot^{-1} (Table 2-III; Figure 2-I). Female pre-pupal development was fastest on plants grown with 1.0 g fertilizer pot^{-1} and slowest on plants grown with 5.0 g fertilizer pot^{-1}. Male pupae developed fastest on plants grown with 0.5 g fertilizer pot^{-1} whereas female pupal development was fastest on plants grown with 1.0 g fertilizer pot^{-1}. For both females and males, overall development from neonate to adult was fastest on plants treated with 1.0 g fertilizer pot^{-1}(Table 2-III). Female larvae consumed similar leaf areas when reared on plants grown with 0.0, 0.5 and 1.0 g fertilizer whereas male larvae consumed more foliage of unfertilized plants than those fertilized at 1.0, 3.0 and 5.0 g pot^{-1} (Table 2-IV). For both female and male specimens, heaviest pupae and silk were produced on plants fertilized at 1.0 g pot^{-1} (Table 2-IV). Adult males and females were heaviest when they were reared as larvae on plants fertilized at 0.5 and 1.0 g pot^{-1} respectively. Moths reared on plants treated with 1.0 g fertilizer pot^{-1} lived longer than those reared on plants treated at any of 0.0, 0.5, 3.0 and 5.0 g pot^{-1}. Largest forewings were produced when specimens were reared on plants fertilized at 1.0 g pot^{-1} (Table 2-V).

For female *P. xylostella* reared on host plants grown under various soil-fertility regimes, a significant positive correlation was found between pupal weight and silk weight ($r = 0.50$, $P < 0.0001$), pupal weight and adult weight ($r = 0.32$, $P = 0.0011$), adult weight and forewing area ($r = 0.22$, $P = 0.0258$), but correlations did not occur between pupal weight and longevity (without food) ($r = 0.17$, $P = 0.0941$), pupal weight and forewing area ($r = 0.22$, $P = 0.2279$), and adult weight and longevity (without food) ($r = 0.02$, $P = 0.8092$). For male specimens, a significant positive correlation was found between pupal weight and silk weight (r = 0.65, P < 0.0001), pupal weight and adult weight (r = 0.55, P < 0.0001), pupal weight and longevity (without food) (r = 0.26, P < 0.0077), and adult weight and longevity (without food) (r = 0.37, P = 0.0002) but no correlation existed between pupal weight and forewing area (r = -0.09, P = 0.3496) and adult weight and forewing area (r = 0.11, P = 0.2620).

Female and male specimens exhibited significant differences in various life history traits when reared on *B. napus* plants grown with the same fertilizer treatment (Table 2-VI). Female pupal development was significantly slower than their male counterparts on plants treated with 1.0 and 3.0 g fertilizer pot^{-1}. Overall development from neonate to adult was faster for females than males when reared as larvae on plants treated with 1.0 g fertilizer pot^{-1}, but development time did not differ for females and males on any other tested fertilizer treatment. Female larvae consumed more leaf tissue than male larvae of plants fertilized at 0.5 and 1.0 g pot^{-1}. Females lived longer than males in the absence of food when they were reared as larvae on plants fertilized at 0.0 and 5.0 g pot^{-1}. In addition, females had larger forewings than males when reared on plants grown with 0.5 and 5.0 g fertilizer pot^{-1} (Table 2-VI).

2.3.3 Plant Parameters

2.3.3.1 Stem Diameter and Numbers of Leaves

Fertilizer treatments strongly affected plant basal stem diameter ($F_{4,36}$ = 13.05, P < 0.0001), and the stem diameter 10 cm above the soil-stem interface ($F_{4,36}$ = 51.34, P < 0.0001) (Table 2-VII). At the soil-stem interface, stem diameter was similar for plants grown under 0.5, 1.0, 3.0 and 5.0 g pot^{-1}. Plants subjected to 3.0 and 5.0 g fertilizer per pot had the largest stem diameter at 10 cm above the soil-stem interface (Table 2-VII).

Mean numbers of leaves per plant varied significantly across different fertilizer applications ($F_{4,36} = 22.64$, $P < 0.0001$). Plants treated at 5.0 g pot^{-1} had the most leaves whereas fewest leaves occurred on plants grown without any added fertilizer (Table 2-VII).

2.3.3.2 Tissue Nutrient Analysis

Soil fertility treatments strongly influenced foliar nutrients (nitrogen: $F_{4,16} = 586.49$, $P < 0.0001$; phosphorous: $F_{4,16} = 122.84$, $P < 0.0001$; potassium: $F_{4,16} = 1081.75$, $P < 0.0001$; sulfur: $F_{4,16} = 667.07$, $P < 0.0001$; calcium: $F_{4,16} = 292.30$, $P < 0.0001$; magnesium: $F_{4,16} = 88.58$, $P < 0.0001$, and sodium: $F_{4,16} = 51.33$, $P < 0.0001$) in *B. napus* (Table 2-VIII). Nutrient content also varied in leaf tissues infested by *P. xylostella* (nitrogen: $F_{4,16} = 2328.83$, $P < 0.0001$; phosphorous: $F_{4,16} = 426.95$, $P < 0.0001$; potassium: $F_{4,16} = 323.55$, $P < 0.0001$; sulfur: $F_{4,16} = 189.78$, $P < 0.0001$; calcium: $F_{4,16} = 3275.15$, $P < 0.0001$; magnesium: $F_{4,16} = 123.41$, $P < 0.0001$, and sodium: $F_{4,16} = 22.52$, $P < 0.0001$) (Table 2-VIII). Leaf nitrogen content increased with fertilizer application rate both for infested and uninfested leaves, but mean nitrogen concentrations of infested leaves consistently remained lower than those of their uninfested counterparts for each application rate (Tables 2-VIII, 2-IX; Figure 2-I). The highest levels of phosphorous occurred in plants grown under 1.0 g, and these differed significantly from the controls and the other treatment plants. No significant differences were observed in phosphorous content between infested and uninfested plants at fertilizer application rates of 0.5 and 1.0 g. Potassium content was significantly different between infested and uninfested plants grown under 0.5, 1.0 and 3.0 g. The highest potassium content was present in infested plants grown at 1.0 g while lowest was present in infested plants grown at 5.0 g (Tables 2-VIII, 2-IX). Under each fertility regime, sulfur content was significantly higher in infested plants than uninfested plants. Leaf sulfur content declined with an increase in fertilizer application rate, with the highest levels found in infested unfertilized plants while the lowest content was in uninfested plants fertilized at 5.0 g pot^{-1} (Tables 2-VIII, 2-IX).

Calcium levels were significantly different between infested and uninfested plants at each fertility treatment; calcium was higher in infested plants grown under 0.0, 0.5 and 1.0 g while lower in infested plants grown under 3.0 and 5.0 g than uninfested plants. Among infested plants, calcium was highest in plants grown at 0.5 g fertilizer pot^{-1} and those that were grown without any added fertilizer; among uninfested plants, calcium was highest in plants grown under 1.0 g (Tables 2-VIII, 2-IX). Magnesium content did not differ significantly between infested and uninfested plants at different tested fertilizer applications except 3.0 and 5.0 g fertilizer pot^{-1} in which magnesium content in infested plants was less than in uninfested plants. Among intact and uninfested plants, the highest magnesium content was present in unfertilized plants while plants treated with 5.0 g had the lowest concentrations (Tables 2-VIII, 2-IX). Sodium content differed significantly between infested and uninfested plants grown without any added fertilizer while there was no significant difference between infested and uninfested plants grown under 0.5, 1.0, 3.0 and 5.0 g fertilizer pot^{-1}. Sodium concentrations in uninfested plants fertilized at 0, 0.5 and 1.0 g were not significantly different; however, plants fertilized at 5.0 g had significantly less sodium compared with unfertilized plants. In infested plants, sodium content was highest in plants that received 3.0 g fertilizer but was not significantly different from those grown under 1.0 g. The lowest sodium content was present in infested unfertilized plants and not significantly different from those that received 5.0 g fertilizer (Tables 2-VIII, 2-IX).

2.3.3.3 Root Masses

Effects of fertility on root mass production (g dry weight) were highly significant when plants were infested and not infested by *P. xylostella* larvae ($F_{4,16} = 346.76$, $P < 0.0001$ and $F_{4,16} = 132.34$, $P < 0.0001$, respectively) (Table 2-X). At each fertility treatment, the root mass was significantly different for infested and uninfested plants; infested plants had larger root masses than their uninfested counterparts. Among intact plants, plants grown with 3.0 g fertilizer pot^{-1} produced more robust root systems compared with all other treatments. When infested, plants grown under 1.0 g fertilizer pot^{-1} had a significantly more robust root system compared with the other treatments. Root masses of infested plants fertilized at 0.5 g pot^{-1} did not differ significantly from unfertilized plants (Table 2-X).

2.4 Discussion

This is the first rigorous study to investigate both bottom-up effects of soil fertility on various life history traits of *P. xylostella*, in addition to top-down effects of its herbivory on host plant nutrient contents, and plant compensatory response. Different fertilizer applications significantly affected the nutrient contents of *B. napus* foliage, and this in turn affected preference and performance of *P. xylostella* (Figures 2-I and 2-II).

Females discriminated among host plants subjected to different levels of soil fertility for oviposition, and tended to select plants on which pre-imaginal survival and development of their offspring was maximal, and on which new generation adults had highest longevity when their food was limited. Plants subjected to herbivory by *P. xylostella* responded by producing elevated levels of some nutrients (e.g., sulfur), but other nutrient levels declined in infested leaves (e.g., nitrogen). Regardless of fertility rate, plants responded to herbivory by increasing root mass compared to uninfested plants. Evidently natural selection in this system has favored the development of physiological mechanisms in female moths to promote selection of those plants that confer greatest potential fitness to their offspring, and has also favored compensatory responses by its host to counteract herbivory.

Among the different nutrients assessed, nitrogen and sulfur appeared to show the greatest effects of herbivory: nitrogen levels declined in infested leaves, but levels of sulfur tended to increase when plants were under attack by herbivores. It is likely that plants redirected some of their nitrogen budget to their defense battery when infested, and this response may have functioned to deprive the herbivore of the essential building blocks for protein synthesis. Sulfur is an important component of the defense system (glucosinolate-myrosinase) in Brassicaceae (Mithen, 1992; Rask et al., 2000; Sarfraz et al. 2006; Chapter 1), and its elevated concentrations in stressed, infested plants suggest that herbivory stimulated enhanced sulfur uptake to better facilitate a defense response by *B. napus*. Greater root mass development in infested plants (Table 2-VII) could enable more efficient extraction from the soil of elements like sulfur that are required in the defense response by plants. Other researchers have provided evidence that plants under biotic stress encounter a trade-off between resource allocation for growth and defense (Rhoades, 1979; Herms and Mattson, 1992). Similar to my present findings, Inbar et al. (2001) reported that nutrient-deficient tomato plants exhibited elevated levels of defensive compounds such as phenolics. Other plant nutrients, particularly potassium and calcium, also exhibited significant responses to *P. xylostella* herbivory in the present study. Are they involved in plant defense or in plant compensation? Their role in this context is unknown and I do not have any plausible explanation for these findings.

Females of *P. xylostella* discriminated between fertilized and unfertilized *B. napus* plants and these findings agree with other reports. For instance, fertilized plants were selected more frequently by ovipositing *Pieris rapae* (L.) (Lepidoptera: Pieridae) females than unfertilized plants (Myers, 1985). In a free-choice test, female *Pieris rapae crucivora* Boisduval deposited approximately five times more eggs on fertilized than on unfertilized cabbage plants (Chen et al., 2004). Females of the leaf beetle, *Cassida canaliculata* Laich. (Coleoptera: Chrysomelidae), deposited significantly more eggs on host plants (*Salvia pratensis* L.) containing high leaf nitrogen content than plants with low nitrogen content (Heisswolf et al., 2005). However, some reports have indicated that females do not distinguish between fertilized and unfertilized plants. For example, female copper butterflies, *Lycaena tityrus* Poda (Lepidoptera: Lycaenidae), did not discriminate between fertilized and unfertilized *Rumex acetosa* L. (Polygonaceae) plants for oviposition; slightly more eggs were deposited on unfertilized than fertilized plants but the effect of host plant quality was not significant (Fischer and Fiedler, 2000).

Although several previous studies have examined effects of insect herbivores on host plant nutrient levels by varying only nitrogen (e.g., Fischer and Fiedler, 2000; Chau and Heinz, 2006), nitrogen content alone does not always explain ovipositional preferences by females, particularly for crucifer specialists such as *P. xylostella* that rely on additional stimulants for host plant selection. In *P. xylostella,* sulfur-deficient plants were less attractive for egg deposition than plants grown with maximal sulfur fertilization (Gupta and Thorsteinson, 1960; Marazzi et al., 2004). Oviposition was significantly higher on sulfur-fertilized *Brassica* species than on sulfur-deficient controls (Gupta and Thorsteinson 1960). Similarly, numbers of eggs laid on artificial leaves treated with the wax-free methanolic leaf-surface extracts of plants grown under normal sulfur (= field concentrations) were significantly higher (ca. 250%) than on artificial leaves sprayed with sulfur-free plant extracts (Marazzi et al., 2004). The present data demonstrate that among fertilized plants, females preferred oviposition on plants containing highest concentrations of phosphorous, sulfur and calcium, and intermediate concentrations of nitrogen, potassium and magnesium (Tables 2-I, 2-VIII).

In the present study, larval and pupal development was slower on plants fertilized at rates of 0.0 and 5.0 g pot^{-1} than on plants grown at 1.0 g pot^{-1}. Contrary to the present findings, higher nitrogen levels increased developmental rates in certain lepidopterans (Slansky and Feeny, 1977; Tabashnik, 1982; Taylor, 1984; Myers, 1985; Cates et al., 1987; Estiarte et al., 1994; Hunter and McNeil, 1997; Fischer and Fiedler, 2000; Chen et al., 2004) and coleopterans (Ohmart et al., 1985; Obermaier and Zwölfer, 1999). In my research, larval and pupal survival was lowest (34.0 and 63.3% respectively) on plants fertilized at 5.0 g pot^{-1} (ca. 10% total nitrogen dry weight basis) and these findings are contradictory to earlier reports in which low nitrogen levels resulted in poor larval survival (Myers and Post, 1981; Taylor, 1984; Myers, 1985; Cates et al., 1987). Similar to my results, significantly higher mortality of *L. tityrus* occurred on plants with nitrogenous fertilizer than on unfertilized plants (Fischer and Fiedler, 2000). Survival in response to fertilization could be species-specific. For instance, survival of two species of grasshoppers varied with low and high nitrogen levels in leaves. Survival of *Phoetaliotes nebrascensis* (Thomas) (Orthoptera: Acrididae) was greatest at low nitrogen levels and decreased in a significant linear fashion as nitrogen levels increased. In contrast, nitrogen levels in diets did not have any effects on survival of *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) (Joern and Behmer, 1998).

Increased nitrogen and other nutrients can lead to unbalanced amino acid profiles and large concentrations of organic acids in plant tissues (Williams and Cronin, 2004), consequently such diets can be detrimental and even toxic to insects (Reese, 1979; Brodbeck et al., 1990). Larvae consumed more foliage of unfertilized plants in the present study. Similarly, *P. rapae* larvae defoliated *Brassica nigra* (L.) plants more than twice as much at low soil fertility compared to high (48.2 and 21.0%, respectively) (Meyer, 2000). There is substantial evidence that insects may have to compensate for lowered nitrogen concentrations by increasing food intake or concentrating their feeding on the most nitrogen-rich parts of plants (Slansky and Feeny, 1977; Tabashnik, 1982; White, 1984; Ravenscroft, 1994; Lavoie and Oberhauser, 2004; Berner et al., 2005). In the present study, plants grown under intermediate fertilizer treatments yielded heavier pupae and greater silk weight. Similarly, heavier pupae of *Coenonympha pamphilus* L. (Lepidoptera: Satyridae) were obtained on fertilized *Festuca rubra* L. (Poaceae) plants than on unfertilized plants (Mevi-Schütz et al., 2003). *Lasiommata megera* L. (Lepidoptera: Satyridae) produced heavier pupae on fertilized plants than on unfertilized

Molinia caerulea (L.) Moench (Poaceae) plants (Bink and Siepel, 1996). Contrary to the present findings, Chen et al. (2004) reported that pupal weight of *P. rapae crucivora* did not differ significantly between insects fed on fertilized and unfertilized *Brassica oleracea* var. *capitata* L. (Brassicaceae) plants. Pupal weight is often considered a good indicator of fecundity (Danathanarayana, 1975; Gilbert, 1984; Nylin and Janz, 1999). In the present study, forewing areas were largest for insects fed on plants fertilized at 1.0 g pot^{-1}. Contrary to my results, host plant nutritional quality did not exhibit any significant effects on wing lengths of *P. rapae* (Chen et al., 2004).

In the present study, females reared as larvae on plants grown at intermediate soil fertility levels lived longer without food suggesting that they obtained better food reserves as larvae. The adaptive advantage of selecting plants of maximal quality may be to confer greater fitness for new generation adults by increasing their longevity when food is limiting, increasing their wing areas, and increasing egg production. Adults of *P. xylostella* are highly migratory (Chapman et al., 2002), and these effects should enhance the success of migration and recolonization, enabling them, for example, to travel in air currents without food over several days from northern Mexico to western Canada (Dosdall et al., 2004b), and then recolonize canola fields in Canada.

Plants that received 1.0 and 3.0 g fertilizer per pot developed more robust root systems (dry weight basis) when plants were infested by *P. xylostella* larvae than when plants were not infested. This concurs with earlier findings in which turnip plants (*B. rapa* L.) infested with 20 larvae of *P. xylostella* had higher root dry weights than plants infested with fewer larvae (Taylor and Bardner, 1968) (see also Chapter 3). Plants can also increase their net aboveground productivity when infested (Belsky, 1986 and references therein) and various mechanisms have been identified that could contribute to such compensatory growth. They include intrinsic mechanisms such as photosynthetic enhancement and reallocation of available assimilates (Delting et al., 1979; Oesterheld and McNaughton, 1988, 1991; Trumble et al., 1993), and extrinsic mechanisms such as nutrient recycling (Floate, 1981; Holland et al., 1992). Therefore, increased shoot growth could lead to increased root growth (McNaughton et al., 1998), and well nourished plants have a higher capability to compensate (and even overcompensate) than when nutrients are limiting. Although I did not continue this experiment to seed production, I suspect that plants grown under intermediate fertility would produce better yields when infested as their robust root systems could ensure better ability to replace the tissues lost to herbivores. Meyer (2000) also noted that at high soil fertility, damaged plants were able to maintain leaf growth rates to a much greater extent during herbivore (*P. rapae*) feeding. Plants grown at ambient soil fertility were able to compensate through increased root and shoot growth and the present results support the compensatory continuum model of Maschinski and Whitham (1989). According to this model, plants will be more likely to compensate for herbivore damage under fertile conditions, because greater nutrient availability will increase plant growth rates and allow plants to replace tissues lost to herbivores more efficiently. There is considerable evidence in support of my results that plants are better able to compensate for damage at higher levels of soil fertility in other systems (Maschinski and Whitham, 1989; Chapin and McNaughton, 1989; Willis et al., 1995; Meyer, 2000; Dosdall et al., 2004a) although I could not find any study on root development in response to *B. napus* fertility and *P. xylostella* herbivory.

Table 2-I. Mean (± S.E.) oviposition by *Plutella xylostella* females in free-choice situations in the greenhouse (intact plant study) (n = 20) and growth chamber (leaf disc study) (n = 10); *Brassica napus* plants were grown under different fertilizer regimes.

	Mean numbers of eggs	
Fertilizer application rate (g pot^{-1})	Intact plants	Leaf discs
0.0	83.10 ^{d} (3.49)	6.80 ^{d} (1.01)
0.5	133.70 ^{b} (5.72)	21.80 ^{b} (1.79)
1.0	180.10 ^{a} (6.95)	28.70 ^{a} (2.20)
3.0	109.10 ^{c} (2.87)	14.90 ^{c} (2.18)
5.0	70.20 ^{d} (5.43)	8.10 ^{d} (1.03)

Means in a column followed by the same letter do not differ significantly (*P* = 0.05) using analysis of variance and Tukey's studentized range test

Table 2-II. Mean (± S.E.) percent survival of *Plutella xylostella* (n = 10) from neonate to pupa and pupa to adult on intact *Brassica napus* plants grown under different fertilizer regimes.

	% Survival	
Fertilizer application rate (g pot^{-1})	Neonate to pupa	Pupa to adult
0.0	50.00 ^{bc}	89.33 ^{a}
	(5.48)	(4.40)
0.5	72.00 ^{a}	97.50 ^{a}
	(3.74)	(2.50)
1.0	76.00 ^{a}	92.42 ^{a}
	(4.00)	(3.13)
3.0	58.00 ^{ab}	84.29 ^{a}
	(3.74)	(6.97)
5.0	34.00 ^{c}	63.33 ^{b}
	(6.78)	(3.33)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 2-III. Mean (± S.E.) developmental times of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Larva to pre-pupa (days)	Female	7.60^{a}	6.80^{b}	6.40^{b}	6.50^{b}	7.60^{a}
		(0.18)	(0.17)	(0.15)	(0.15)	(0.26)
	Male	7.40^{a}	6.90^{a}	5.90^{b}	6.00^{b}	7.30^{a}
		(0.15)	(0.19)	(0.07)	(0.07)	(0.31)
Pre-pupa to pupa (days)	Female	1.10^{ab}	1.00^{ab}	0.90^{b}	1.10^{ab}	1.30^{a}
		(0.07)	(0.00)	(0.05)	(0.07)	(0.14)
	Male	0.95^{a}	0.80^{a}	1.00^{a}	1.05^{a}	1.00^{a}
		(0.03)	(0.06)	(0.09)	(0.08)	(0.09)
Pupa to adult (days)	Female	4.50^{a}	4.20^{a}	3.10^{b}	4.00^{a}	4.50^{a}
		(0.15)	(0.14)	(0.22)	(0.23)	(0.26)
	Male	5.10^{a}	4.10^{b}	4.50^{ab}	4.50^{ab}	5.10^{a}
		(0.24)	(0.19)	(0.18)	(0.21)	(0.19)
Larva to adult (days)	Female	13.20^{a}	12.00^{b}	10.40^{c}	11.60^{b}	13.40^{a}
		(0.25)	(0.25)	(0.32)	(0.29)	(0.35)
	Male	13.45^{a}	11.80^{b}	11.40^{b}	11.55^{b}	13.40^{a}
		(0.32)	(0.35)	(0.26)	(0.23)	(0.37)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 2-IV. Mean (± S.E.) foliage consumption, pupal weights and silk weights of *Plutella xylostella* female (n = 20) and male (n =20) specimens when larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Foliage consumed (cm^2)	Female	2.74 ^{a}	2.85 ^{a}	3.13 ^{a}	1.82 ^{b}	1.83 ^{b}
		(0.14)	(0.25)	(0.21)	(0.25)	(0.08)
	Male	2.28 ^{a}	2.06 ^{ab}	1.78 ^{b}	1.70 ^{b}	1.73 ^{b}
		(0.13)	(0.12)	(0.19)	(0.04)	(0.11)
Pupal weight (mg)	Female	6.46 ^{bc}	6.86 ^{abc}	7.67 ^{a}	7.25 ^{ab}	6.36 ^{c}
		(0.15)	(0.25)	(0.22)	(0.21)	(0.18)
	Male	5.90 ^{c}	6.54 ^{ab}	6.71 ^{a}	6.38 ^{abc}	6.02 ^{bc}
		(0.21)	(0.21)	(0.14)	(0.12)	(0.17)
Silk weight (mg)	Female	0.191 ^{ab}	0.204 ^{ab}	0.236 ^{a}	0.221 ^{a}	0.174 ^{b}
		(0.006)	(0.010)	(0.006)	(0.017)	(0.018)
	Male	0.178 ^{c}	0.213 ^{bc}	0.263 ^{a}	0.232 ^{ab}	0.190 ^{c}
		(0.011)	(0.013)	(0.006)	(0.005)	(0.012)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 2-V. Mean (± S.E.) adult body weights, longevity without food and forewing areas of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Adult body weight (mg)	Female	2.62^{ab}	2.49^{b}	2.99^{a}	2.54^{b}	2.84^{ab}
		(0.10)	(0.07)	(0.14)	(0.11)	(0.10)
	Male	2.19^{c}	2.61^{bc}	3.07^{ab}	3.09^{a}	2.57^{c}
		(0.11)	(0.12)	(0.11)	(0.14)	(0.12)
Longevity without food (days)	Female	6.70^{b}	6.70^{b}	7.90^{a}	7.00^{b}	7.10^{b}
		(0.18)	(0.18)	(0.19)	(0.18)	(0.28)
	Male	5.60^{c}	6.20^{c}	7.70^{a}	7.00^{b}	6.10^{c}
		(0.11)	(0.25)	(0.15)	(0.23)	(0.19)
Forewing area (cm^2)	Female	0.167^{b}	0.177^{ab}	0.196^{a}	0.184^{ab}	0.191^{a}
		(0.003)	(0.004)	(0.006)	(0.004)	(0.006)
	Male	0.158^{bc}	0.150^{c}	0.185^{a}	0.174^{ab}	0.145^{c}
		(0.008)	(0.003)	(0.004)	(0.002)	(0.005)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 2-VI. Pair-wise comparisons (*t*-values) between female (n = 20) and male (n = 20) *Plutella xylostella* for some key life history parameters when larvae were reared on *Brassica napus* grown under similar fertility treatment.

Biological parameters	Fertilizer application rate (g pot^{-1})				
	0.0	0.5	1.0	3.0	5.0
Development time (larva to pre-pupa)	0.84 ^{ns}	-0.39 ^{ns}	2.99**	2.94**	0.75 ^{ns}
Development time (pre-pupa to pupa)	1.95 ^{ns}	3.56**	-1.00 ^{ns}	0.47 ^{ns}	1.83 ^{ns}
Development time (pupa to adult)	-2.11*	0.43 ^{ns}	-4.92***	-1.60 ^{ns}	-1.88 ^{ns}
Development time (larva to adult)	-0.62 ^{ns}	0.46 ^{ns}	-2.44*	0.13 ^{ns}	0.00 ^{ns}
Herbivory (per larva)	2.38*	4.73***	4.83***	0.43 ^{ns}	0.74 ^{ns}
Pupal weight	2.16*	0.98 ^{ns}	3.71***	3.65***	1.39 ^{ns}
Silk weight	1.03 ^{ns}	-0.53 ^{ns}	-3.05**	-0.65 ^{ns}	-0.79 ^{ns}
Adult body weight	2.84**	-0.83 ^{ns}	-0.45 ^{ns}	-3.20**	1.71 ^{ns}
Adult longevity (without food)	5.20***	1.64 ^{ns}	0.83 ^{ns}	0.00 ns	2.95**
Forewing area	0.90 ^{ns}	5.60***	1.47 ^{ns}	2.54*	5.67***

^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

Table 2-VII. Mean (± S.E.) stem diameter and numbers of leaves of *Brassica napus* plants (n = 10) grown under different fertilizer regimes.

	Stem diameter (mm)		Numbers of leaves per plant
Fertilizer application rate (g pot^{-1})	Soil-stem interface	10 cm above the soil-stem interface	
0.0	6.30^{b}	7.15^{c}	9.20^{d}
	(0.25)	(0.33)	(0.36)
0.5	8.09^{a}	8.93^{b}	14.30^{c}
	(0.22)	(0.24)	(0.70)
1.0	8.93^{a}	9.86^{b}	14.40^{bc}
	(0.32)	(0.26)	(0.76)
3.0	8.94^{a}	11.86^{a}	17.30^{ab}
	(0.40)	(0.22)	(1.00)
5.0	8.80^{a}	11.61^{a}	17.10^{a}
	(0.33)	(0.33)	(0.50)

Means in a column followed by the same letter do not differ significantly (P = 0.05) using analysis of variance and Tukey's studentized range test

Table 2-VIII. Mean (± S.E.) nutrients in leaf tissue of *Brassica napus* grown under different fertilizer regimes when infested (n = 15) and not infested (intact) (n = 15) by *Plutella xylostella* larvae.

Leaf tissue nutrients (%)		Fertilizer application rate (g pot^{-1})				
		0.0	0.5	1.0	3.0	5.0
Nitrogen	Intact	4.180 ^{e} (0.107)	6.502 ^{d} (0.031)	7.106 ^{c} (0.024)	8.648 ^{b} (0.165)	10.382 ^{a} (0.081)
	Infested	3.102 ^{d} (0.026)	5.518 ^{c} (0.046)	5.532 ^{c} (0.083)	7.448 ^{b} (0.041)	9.312 ^{a} (0.024)
Phosphorous	Intact	0.228 ^{c} (0.009)	0.766 ^{b} (0.009)	1.116 ^{a} (0.060)	0.860 ^{b} (0.007)	0.826 ^{b} (0.009)
	Infested	0.286 ^{d} (0.016)	0.750 ^{c} (0.018)	1.078 ^{a} (0.007)	0.928 ^{b} (0.016)	0.928 ^{b} (0.010)
Potassium	Intact	6.102 ^{c} (0.026)	6.660 ^{a} (0.009)	6.148b ^{c} (0.007)	6.196 ^{b} (0.012)	5.342 ^{d} (0.007)
	Infested	6.146 ^{c} (0.044)	7.480 ^{b} (0.023)	8.362 ^{a} (0.069)	7.340 ^{b} (0.087)	5.286 ^{d} (0.087)
Sulfur	Intact	2.058 ^{b} (0.017)	1.846 ^{c} (0.014)	2.170 ^{a} (0.011)	1.324 ^{d} (0.022)	1.162 ^{e} (0.010)
	Infested	3.330 ^{a} (0.122)	2.724 ^{b} (0.017)	2.528 ^{b} (0.017)	1.546 ^{c} (0.017)	1.560 ^{c} (0.037)
Calcium	Intact	2.166 ^{b} (0.042)	1.932 ^{c} (0.012)	2.550 ^{a} (0.033)	2.016 ^{c} (0.005)	1.106 ^{d} (0.042)
	Infested	3.648 ^{a} (0.027)	3.662 ^{a} (0.021)	2.782 ^{b} (0.021)	1.778 ^{c} (0.026)	0.918 ^{d} (0.021)
Magnesium	Intact	0.888 ^{a} (0.024)	0.626 ^{bc} (0.012)	0.678 ^{b} (0.015)	0.550 ^{c} (0.015)	0.392 ^{d} (0.020)
	Infested	0.918 ^{a} (0.020)	0.682 ^{b} (0.026)	0.630 ^{b} (0.019)	0.382 ^{c} (0.023)	0.328 ^{c} (0.007)
Sodium	Intact	0.340 ^{a} (0.018)	0.330 ^{a} (0.013)	0.334 ^{a} (0.011)	0.290 ^{b} (0.014)	0.240 ^{c} (0.017)
	Infested	0.196 ^{c} (0.016)	0.286 ^{b} (0.017)	0.328 ^{ab} (0.020)	0.350 ^{a} (0.017)	0.212 ^{c} (0.012)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 2-IX. Pair-wise comparison (*t*-values) for nutrients in *Brassica napus* leaf tissue grown under different fertilizer regimes when infested and not infested by *Plutella xylostella* larvae.

Leaf tissue nutrients (%)	Fertilizer application rate (g pot^{-1})				
	0.0	0.5	1.0	3.0	5.0
Nitrogen	-9.82***	-17.59***	-18.16***	-7.06***	-12.66***
Phosphorous	3.19*	-0.81 ^{ns}	-0.62 ^{ns}	3.97**	7.60***
Potassium	0.86 ^{ns}	33.20***	31.86***	13.07***	-0.64 ^{ns}
Sulfur	10.32***	39.19***	17.59***	7.79***	10.26***
Calcium	29.41***	72.59***	5.92***	-8.93***	-4.03**
Magnesium	0.97 ^{ns}	1.96 ^{ns}	-1.97 ^{ns}	-6.16***	-3.02*
Sodium	-6.05***	-2.06 ^{ns}	-0.26 ^{ns}	2.68*	-1.36 ^{ns}

^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

Table 2-X. Mean (± S.E.) root mass development in *Brassica napus* plants grown under different fertilizer regimes when infested (n = 5) and not infested (intact) (n = 5) by *Plutella xylostella* larvae.

	Root mass (gram dry weight)		
Fertilizer application rate (g pot^{-1})	Intact plants	Infested plants	Infested vs. Intact
0.0	0.2250 ^{d} (0.005)	0.3212 ^{d} (0.0068)	$t = 14.13$; $P < 0.0001$
0.5	0.2678 ^{d} (0.0054)	0.3526 ^{d} (0.0102)	$t = 7.35$; $P < 0.0001$
1.0	0.4284 ^{b} (0.0173)	0.8506 ^{a} (0.0073)	$t = 22.45$; $P < 0.0001$
3.0	0.5040 ^{a} (0.0184)	0.6648 ^{b} (0.0035)	$t = 8.58$; $P < 0.0001$
5.0	0.3516 ^{c} (0.0043)	0.4994 ^{c} (0.0197)	$t = 7.33$; $P < 0.0001$

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and

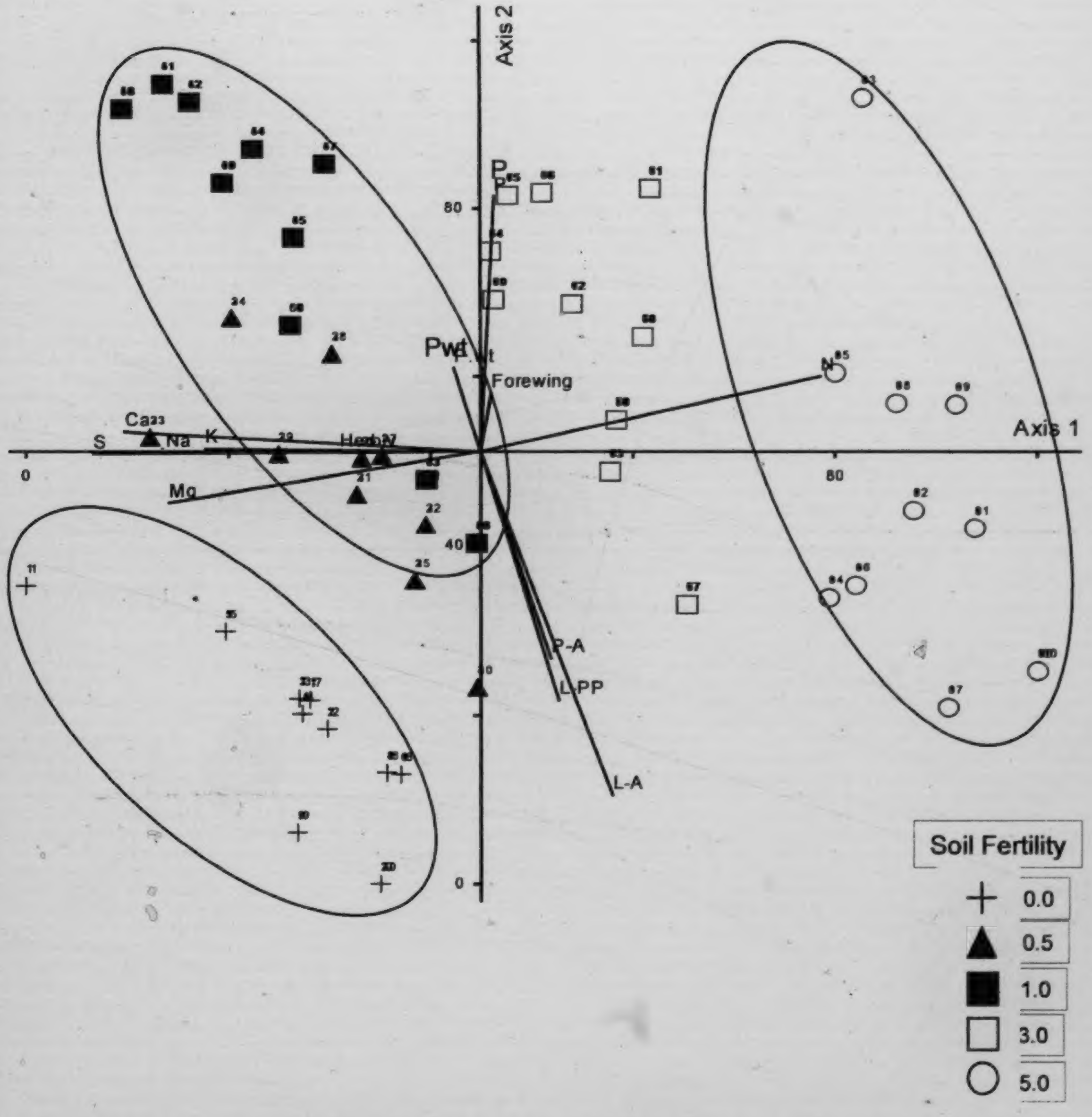

Figure 2-I. Multivariate/ordination analysis: Soil fertility, foliage nutrients and female *Plutella xylostella* fitness correlates (N = nitrogen, P = phosphorous, K = potassium, S = sulfur, Ca = calcium, Mg = magnesium, Na = sodium, L-PP = larva to pre-pupa, P-A = pupa to adult, L-A = larva to adult, Pwt = pupal weight).

	***Brassica napus* Host, without Herbivory**	***Brassica napus* Host, with Herbivory**	***Plutella xylostella* - Host Plant Interaction**
High Fertility	• large stem diameter • high leaf number • high leaf N content, but low or intermediate P, K, S, Ca, Mg, Na • intermediate root biomass	• large stem diameter • high leaf number • high leaf N, intermediate P, low K, S, Ca, Mg, Na contents • intermediate root biomass	• few eggs deposited • low larval survival • slow larval/pupal development • low pupal weight • small adult wing area • short-lived adults
Intermediate Fertility	• large stem diameter • intermediate leaf number • intermediate leaf N and Mg contents, high P, K, S, Ca, Mg, and Na contents • high root biomass	• large stem diameter • intermediate leaf number • intermediate leaf N, S, Ca and Mg contents, high P, K, high P, K, S, Ca, Mg, Na contents • high root biomass	• most eggs deposited • highest larval survival • fast larval/pupal development • high pupal weight • large adult wing area • long-lived adults
Low Fertility	• small stem diameter • low leaf number • low leaf N and P contents, intermediate S, Ca contents, high Mg, Na contents • low root biomass	• small stem diameter • low leaf number • low leaf N, P, Na contents, intermediate K content, and high S, Ca, and Mg contents • low root biomass	• few eggs deposited • high larval survival • slow larval/pupal development • low pupal weight • small adult wing area • short-lived adults

Figure 2-II. Effects of different fertility regimes on *Brassica napus* plants without and with herbivory, and *Plutella xylostella* -host plant interactions.

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Chapter 3:

Resistance of Some Cultivated Brassicaceae to Infestations by *Plutella xylostella* [‡]

3.1 Introduction

Host plant resistance has been used effectively in sustainable integrated management programs for several crop pests (Dosdall et al., 1994, 2000; Dent, 2000; Raza et al., 2000; Sarfraz et al., 2003, 2006). van Emden (1991) suggested that insecticide concentrations could be reduced three-fold on resistant host plants without appreciable increases in the pest population. Plant resistance can occur through one or a combination of factors involving antibiosis, antixenosis, and tolerance (Painter, 1951). Plant characteristics responsible for antibiosis may cause reduced insect survival, decreased size or weight, reduced longevity and reproduction in new generation adults, or have an indirect effect by increasing the exposure of the insect to its natural enemies as a result of prolonged developmental time (Dent, 2000). Plants that exhibit antixenosis would have reduced initial infestations or a higher emigration rate of the pest than their susceptible counterparts (Teetes, 1996; Dent, 2000; Sarfraz et al., 2006). Tolerance includes plant responses that minimize the effects of herbivory on the fitness of individual plants (Simms, 2000; Tiffin, 2000). Herbivores can elicit different physiological responses in infested plants that would not usually occur in uninfested individuals. Such 'induced' or 'active' tolerance can then lead to compensatory growth of plants as a result of various mechanisms including enhancement of photosynthetic activity, activation of dormant meristems, and utilization of stored reserves (Trumble et al., 1993; Tiffin, 2000).

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Herbicide-tolerant crops have revolutionized agricultural production systems. Several varieties of herbicide-resistant canola, *Brassica napus* L. (Brassicaceae), have been widely planted in the past several years in Canada. It is generally assumed that proteins conferring herbicide tolerance should not affect insects, but no study has compared the life history parameters of any insect pest on conventional and herbicide-tolerant plants.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive insect pest of brassicaceous crops worldwide. It globally requires over US$1.0 billion in estimated annual management costs (Talekar and Shelton, 1993). It occurs annually throughout the prairie provinces of Canada wherever brassicaceous crops are grown and can cause substantial crop losses during outbreak years (Dosdall et al., 2004). Under intensive selection pressure, insects often develop high levels of insecticide resistance and *P. xylostella* is one of the "leaders" among insect pests that are very difficult to control (Mota-Sanchez et al., 2002; Sarfraz and Keddie, 2005). Host plant resistance, therefore, can serve as an important tool for *P. xylostella* management with reduced input of insecticides. However, a review of the literature indicates that extensive studies on plant resistance against *P. xylostella* are rare. The majority of research has focused on comparing selected cultivars within a species (e.g., Hamilton et al., 2005), without providing complete details on life history parameters of *P. xylostella* that are directly related to pest population dynamics. No previous study has compared the compensatory ability of various brassicaceous species and cultivars in the form of root development when infested by *P. xylostella*.

The present study was designed to evaluate eight Brassicaceae commonly available in various crucifer-growing areas worldwide for their resistance against *P. xylostella*. Ovipositional preference (antixenosis) was investigated in a free-choice situation and the potential role of leaf trichome density in antixenosis was determined. For potential antibiotic resistance, several *P. xylostella* life history parameters (i.e., survival, pre-imaginal developmental time, larval herbivory, pupal weight, silk weight, adult bodyweight, forewing area, adult longevity, and oviposition of new generation adults) were investigated on all eight Brassicaceae in no-choice tests. Plant compensatory ability (tolerance) in the form of belowground biomass development following *P. xylostella* herbivory was also assessed. A further objective of this study was to provide detailed insights on some key life history traits of both male and female *P. xylostella* when specimens were reared on various host plants.

3.2 Materials and Methods

3.2.1 Insects and Plants

The laboratory colony of *P. xylostella* was maintained on potted *B. napus* cv. Q2 plants at 22±0.5°C with 16h L: 8h D. Moths collected from different fields in Alberta, Canada were added to the culture every summer to maintain genetic diversity.

Eight Brassicaceae, namely *B. napus* cv. Q2 (susceptible to both glufosinate ammonium and glyphosate herbicides), *B. napus* cv. Liberty (resistant to glufosinate ammonium), *B. napus* cv. Conquest (resistant to glyphosate), *B. rapa* L. cv. Reward, *B. juncea* (L.) Czern. cv. Cutlass, *B. carinata* L. (Accession No. BCA-003), *B. oleracea* L. cv. Red Acre, and *Sinapis alba* L. (Accession No. SAL-004) were grown under greenhouse conditions. Plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium and fertilized with 20: 20: 20 (N: P: K) at 0.5 g pot^{-1} when plants were two to three weeks old. Four-week-old plants were used for all experiments.

3.2.2 Oviposition Choice and Trichome Density

Ovipositional preference experiments were conducted with *P. xylostella* in a free-choice situation in five screened cages (120 x 120 x 120 cm) using a randomized complete block design and each cage was considered a block. One plant from each species and cultivar was placed randomly in each cage and a total of 40 plants were used in the experiment (i.e., eight plants in each cage). Sixteen one-day-old adults (two moths per plant) were released in 1:1 (m: f) sex ratio in the middle of each cage and provided a sterile 10% honey solution for adult feeding. Eggs on each plant were counted 2 d following moth release. Leaf discs (area = 1.0 cm^2) were taken from five leaves of each species and cultivar (one leaf from each replicate plant) and trichome densities on adaxial and abaxial leaf surfaces were determined using a dissecting microscope as described by Raza et al. (2000).

3.2.3 Effects of Host Plants on Insect Life History Traits

3.2.3.1 Whole Plant Study: Survival of *P. xylostella*

Over 100 newly emerged *P. xylostella* were caged and allowed to oviposit on tinfoil sheets treated with an extract of *B. napus* leaves. After 24 h, egg sheets were collected, and incubated in individual plastic cups. Survival from neonate to pupa was assessed in screened cages (40 x 40 x 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 80 cages with 10 plants from each species and cultivar. Each of five plants from each genotype were infested with ten first-instar larvae while the remaining plants served as uninfested controls. Plants were observed every 48 h and the numbers of surviving insects were recorded. Pupae were harvested, weighed and kept individually in transparent plastic cups until adult emergence.

3.2.3.2 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

This experiment was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D). Excised leaves were placed on moist filter papers (9-cm diameter) in plastic containers; holes were poked in each transparent lid to ensure ventilation and to avoid condensation. For each plant genotype, 100 second-instar larvae (≤ 1 day old) taken from the laboratory colony were introduced into individual plastic containers; a total of 800 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Developmental times from second-instar larva to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were also weighed using a Sartorius Supermicro scale (Sartorius Inc., Edgewood, NY, USA). Adults were separated by sex and twenty pairs were used in the longevity (without food), bodyweight and forewing area experiments, whereas ten pairs were used in the oviposition and longevity (with food) experiments.

To quantify levels of larval feeding, all leaves damaged by *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA) and the final version (250 dpi) was saved as a TIFF file without LZW compression. Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory.

Twenty females and 20 males reared from each plant taxon were used to determine their longevity without food. Moths were weighed within 24 h of their death. Their forewings were carefully removed, glued onto a paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA), and their areas were measured using Image J.

Ten pairs of moths of almost the same age (≤ 1 day old) reared from each plant taxon were released in individual plastic containers (i.e., one pair per cup) containing *B. napus* cv. Q2 leaf discs (area = 52.8 cm^2) placed on moist filter papers. A total of 80 cups were used following a completely randomized design and this experiment was conducted in the growth chamber (22±0.5°C with 16h L: 8h D). Insects were allowed to oviposit over an 8-d period and eggs found on both the leaf disc and off the leaf disc (i.e., filter paper, and walls and lids of containers) were recorded daily. Every 24 h, moths were transferred to new containers and provided with fresh leaf discs and food (sterile 10% honey solution). Every day, the leaf discs were taken from undamaged plants to avoid any induced effects on oviposition by *P. xylostella*. After the eighth day, only females were kept in the containers with a continuous supply of 10% honey solution and observed daily until mortality.

3.2.4 Root Mass Development in Response to Insect Herbivory

At the end of the experiment investigating *P. xylostella* survival on whole plants, infested and uninfested (control) plants were uprooted. The roots were carefully washed, removed from the plants, air-dried at room temperature and weighed to determine the effects of aboveground herbivory on root mass (g dry weight basis) development.

3.2.5 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used as necessary to achieve normality and homoscedasticity before analysis (Steel et al., 1997), but untransformed means are presented. Analysis of variance (ANOVA) (PROC GLM) for a randomized complete block design was performed for oviposition choice experiments with cage as a blocking factor. ANOVA for a completely randomized design was performed as necessary to test the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). Correlation (PROC CORR) was used to contrast oviposition preference and trichome density. Correlations were also determined between pupal weight and silk weight, pupal weight and adult weight, pupal weight and longevity without food, pupal weight and forewing area, adult weight and longevity, and pupal weight and oviposition. T-tests (PROC TTEST) were performed for pair-wise comparison between female and male specimens for their development time, larval herbivory, pupal weight, silk weight, adult bodyweight, forewing area and longevity (without food) when they were reared on various Brassicaceae. PROC TTEST was also performed for pair-wise comparison between root masses of infested and uninfested (control) plants for each plant genotype separately.

3.3 Results

3.3.1 Oviposition Choice and Trichome Density

Plant species and cultivars had significant effects on *P. xylostella* oviposition preference (F = 44.37; df = 7; $P < 0.0001$) (Figure 3-I). Oviposition on *S. alba* and *B. rapa* were similar, but numbers of eggs deposited on *S. alba* exceeded other plant genotypes (Figure 3-I). Females laid 2.8-, 4.2-, and 14.8-fold more eggs on *S. alba* than on *B. napus* cv. Q2, *B. napus* cv. Conquest and *B. oleracea*, respectively. *Brassica juncea*, *B. napus* cv. Liberty and *B. carinata* plants received 1.5-, 1.6-, and 1.9-fold fewer eggs respectively than *S. alba* (Figure 3-I). Mean numbers of eggs deposited on *B. napus* cv. Q2 were statistically similar to those laid on *B. carinata* and *B. napus* cv. Conquest. Among *B. napus* cultivars, females deposited 1.7- and 2.6-fold more eggs on Liberty than on Q2 and Conquest respectively (Figure 3-I).

Trichome density varied significantly on adaxial (F = 95.13; df = 7; $P < 0.0001$) and abaxial (F = 126.12; df = 7; $P < 0.0001$) leaf surfaces among different plant genotypes tested. *Brassica carinata* had the highest number of trichomes followed by *B. juncea* and *B. rapa* (Figure 3-II) while *B. napus* cv. Q2, *B. napus* cv. Liberty, *B. napus* cv. Conquest, *B. oleracea* and *S. alba* lacked trichomes. There was no correlation between oviposition and the overall trichome density ($r = 0.25$; $P = 0.1127$).

3.3.2 Effects of Host Plants on Insect Life History Traits

Survival from neonate to pupa and from pupa to adult was statistically similar on whole plants of all eight Brassicaceae tested (Table 3-I). Host plant genotypes significantly affected larval and pupal development time of females ($F = 20.67$; df = 7; $P < 0.0001$ and $F = 4.50$; df = 7; $P = 0.0002$ respectively) and males ($F = 29.16$; df = 7; $P < 0.0001$ and $F = 9.92$; df = 7; $P < 0.0001$ respectively) (Table 3-II). Pre-pupal development time was significantly affected for males ($F = 2.14$; df = 7; $P = 0.0432$), but not for females ($F = 1.97$; df = 7; $P = 0.0633$) (Table 3-II). Host plants had significant effects on foliage consumption by individual female and male larvae ($F = 7.72$; df = 7; $P < 0.0001$ and $F = 3.12$; df = 7; $P = 0.0045$ respectively) (Table 3-III). Pupal weight and silk weight differed significantly for females ($F = 28.27$; df = 7; $P < 0.0001$ and $F = 10.88$; df = 7; $P < 0.0001$ respectively) and males ($F = 14.08$; df = 7; $P < 0.0001$ and $F = 8.37$; df = 7; $P < 0.0001$ respectively) when reared on various host plant genotypes (Table 3-III). Host plants significantly affected adult body weight, forewing area and longevity (without food) of females ($F = 21.17$; df = 7; $P < 0.0001$, $F = 10.05$; df = 7; $P < 0.0001$ and $F = 31.24$; df = 7; $P < 0.0001$ respectively) and males ($F = 9.20$; df = 7; $P < 0.0001$, $F = 7.00$; df = 7; $P < 0.0001$ and $F = 7.03$; df = 7; $P < 0.0001$ respectively) (Table 3-IV).

Female larval development was fastest on *B. juncea* whereas male larvae developed faster on *B. napus* cv. Liberty than on other plants tested (Table 3-II). Female and male pupae developed fastest on *S. alba* and *B. napus* cv. Conquest respectively (Table 3-II). Both female and male larvae consumed largest leaf area of *B. rapa* whereas male larvae had least foliage consumption on *B. napus* cv. Liberty. Female and male pupae were lighter on *B. napus* cv. Liberty and *B. oleracea* respectively than on other tested plant species and cultivars (Table 3-III). Female specimens reared on *S. alba* produced more silk than those on other host plants tested. Male specimens reared on *B. napus* cv. Conquest produced significantly more silk than those on *B. juncea* and *B. carinata* (Table 3-III). Heaviest females were produced on *S. alba* whereas males were heaviest on *B. napus* cv. Liberty (Table 3-IV). Female moths reared on *B. napus* cv. Conquest had the largest forewings whereas male moths reared on *B. napus* cv. Conquest and *B. oleracea* had the largest forewings (Table 3-IV). Female and male specimens reared on *B. napus* cv. Liberty and *B. oleracea* lived for the shortest time in the absence of adult nutrition; longevity of females reared on the tested Brassicaceae, however, did not differ significantly when food was provided (Table 3-IV). For female specimens reared on various host plant genotypes, a significant correlation was found between pupal weight and silk weight ($r = 0.43$; $P < 0.0001$), pupal weight and adult weight ($r = 0.50$; $P < 0.0001$), pupal weight and longevity (without food) ($r = 0.60$; $P < 0.0001$), pupal weight and forewing area ($r = 0.50$; $P < 0.0001$), and adult weight and longevity (without food) ($r = 0.45$; $P < 0.0001$). For male specimens, a significant correlation was found between pupal weight and silk weight ($r = 0.21$; $P = 0.0078$), pupal weight and adult weight ($r = 0.27$; $P = 0.0007$), pupal weight and longevity (without food) ($r = 0.40$; $P <$

0.0001), and adult weight and longevity (without food) ($r = 0.19$; $P = 0.0168$) but no correlation existed between pupal weight and forewing area ($r = 0.01$; $P = 0.8542$).

Female and male specimens exhibited significant differences in various life history traits when reared on the same host plant genotype (Table 3-V). Female pupal development was significantly faster than their male counterparts on all the crucifers tested in this study. In addition, females were heavier and lived longer than males in the absence of food (Table 3-V).

Over the 8-d oviposition period, females deposited significantly different numbers of total eggs on the leaf discs ($F = 110.96$; df = 7; $P < 0.0001$), total eggs off the leaf discs ($F = 34.74$; df = 7; $P < 0.0001$), and overall eggs (on the discs and off the discs) ($F = 137.37$; df = 7; $P < 0.0001$) when they were raised on the Brassicaceae tested in this study (Table 3-VI). Females reared on *B. napus* cv. Conquest and *B. juncea* deposited the most total eggs (436.80 ± 6.69 and 401.60 ± 7.51 respectively) while females from *B. oleracea* laid the fewest eggs (180.40 ± 6.86) (Table 3-VI). Overall oviposition of females reared on *B. napus* cv. Q2, *B. rapa* and *B. carinata* did not differ significantly (Table 3-VI). Females reared on *B. napus* cv. Conquest exhibited less discrimination between substrates and deposited the most eggs off the leaf disc followed by females raised on *B. juncea* and *S. alba* (Table 3-VI). No correlation was found between oviposition and pupal weight ($r = 0.11$; $P = 0.3130$).

3.3.3 Root Mass Development in Response to Insect Herbivory

Pair-wise comparisons for each genotype indicated that infested *B. napus* cv. Q2 ($t = 7.37$; df = 8; $P < 0.0001$), *B. napus* cv. Conquest ($t = 2.97$; df = 8; $P = 0.0179$), *B.*

rapa ($t = 12.30$; df = 8; $P < 0.0001$), *B. carinata* ($t = 2.99$; df = 8; $P = 0.0174$) and *S. alba* ($t = 15.90$; df = 8; $P < 0.0001$) had significantly more robust root systems than their uninfested counterparts (Figure 3-III). However, the root masses of *B. napus* cv. Liberty ($t = 0.84$; df = 8; $P = 0.4245$), *B. juncea* ($t = 0.44$; df = 8; $P = 0.6703$) and *B. oleracea* ($t = -2.20$; df = 8; $P = 0.0587$) did not differ significantly between infested and uninfested plants (Figure 3-III).

3.4 Discussion

3.4.1 Oviposition Choice and Trichome Density

Sinapis alba was most preferred by *P. xylostella* among the Brassicaceae host plants evaluated and this may be attributed to morphological and chemical properties of the species. For instance, *S. alba* has a glossy phylloplane (Justus et al., 2000), and *P. xylostella* prefers to oviposit on glossy substrates rather than on their non-glossy counterparts (Eigenbrode et al., 1991a, b; Justus et al., 2000; Shelton and Nault, 2004). Furthermore, *S. alba* plants contain higher concentrations of aromatic glucosinolates (mainly hydroxybenzyl and benzyl) whereas alkenyl/aliphatic and indolyl glucosinolates predominate in *Brassica* species (Mewis et al., 2002; Sarfraz et al., 2006). *Plutella xylostella* survival was similar on the Brassicaceae tested (see Section 3.3.2) suggesting that plants like *S. alba* that received more eggs would have higher larval infestations, and this is consistent with earlier reports. For example, significantly more *P. xylostella* larvae were reported on *S. alba* than on *B. juncea* and *B. napus* under field conditions (Brown et al., 1999). By contrast, *S. alba* plants were less susceptible than other Brassicaceae to infestations by crucifer specialists such as the cabbage maggot, *Delia radicum* (L.)

(Diptera: Anthomyiidae) (Dosdall et al., 2000), the flea beetles, *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae) (Lamb, 1984; Bodnaryk and Lamb, 1991; Palaniswamy and Lamb, 1992), and the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) (Brown et al., 1999; Kalischuk and Dosdall, 2004). These differences in susceptibility are perhaps not surprising because different crucifer specialists are known to vary in their responses to different glucosinolates. For instance, indolyl glucosinolates had greater stimulatory effects than aromatic and alkenyl glucosinolates in *Pieris rapae* (L.) (Lepidoptera: Pieridae), *D. radicum*, and *Delia floralis* (Fallén) (Diptera: Anthomyiidae) (Renwick et al., 1992; Simmonds et al., 1994; Roessingh et al., 1997). Some evidence exists that extracts from *S. alba* contained an unknown deterrent for *P. xylostella* oviposition (Reed et al., 1989), but diverse morphologies and chemistries probably override such compounds when whole plants (instead of extracts) of *S. alba* are presented to females along with other Brassicaceae.

Brassica napus cv. Conquest showed higher levels of antixenotic resistance for ovipositing females than *B. napus* cv. Liberty, but *B. napus* cvs. Q2 and Conquest were equally preferred for oviposition by *P. xylostella*. There is considerable evidence that differences in resistance can vary significantly among cultivars within the same species (Dosdall et al., 2000; Hamilton et al., 2005). Trichome density on adaxial and abaxial leaf surfaces did not contribute to this difference in resistance as the *B. napus* cultivars tested lack trichomes (see Section 3.3.1). Further research is required to investigate the mechanisms involved in this antixenosis.

No correlation existed between oviposition and overall trichome density but these results do not support earlier reports. For instance, a negative relationship was observed between trichome density and *P. xylostella* oviposition on *Arabidopsis thaliana* L. (Handley et al., 2005). By contrast, *P. xylostella* oviposition on Chinese cabbage, *Brassica rapa* L. ssp. *pekinensis* (Lour.) cv. New King, was significantly correlated with trichome density (Talekar et al., 1994). It is hypothesized that when a range of plant genotypes with diverse morphologies are provided in a choice situation, trichome density as a factor regulating *P. xylostella* oviposition is not of sole importance.

3.4.2 Effects of Host Plants on Insect Life History Traits

Charleston and Kfir (2000) reported that *P. xylostella* larval survival was significantly lower on *B. juncea* than on cabbage (*B. oleracea* var. *capitata*), broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*) and Chinese cabbage (*B. rapa* ssp. *pekinensis*), and suggested that *B. juncea* could be used as trap crop in cabbage-growing regions. Badenes-Perez et al. (2004) concluded that percentage survival from egg to pupation was higher on *B. juncea* than on *B. oleracea*, but my results indicated that insect survival did not differ among Brassicaceae plants tested. Larval development was faster and females were more fecund on *B. juncea* than on *B. oleracea*, suggesting that *B. juncea* planted along with *B. oleracea* as trap crop could instead serve as a nursery crop for *P. xylostella* in situations where growers are unable to manage pest insects on *B. juncea* in time. Measuring defoliation is a useful tool for evaluating host-plant resistance (Jansky et al., 1999), but no previous study has compared *P. xylostella* herbivory on various Brassicaceae. Digital analyses demonstrated that *B. rapa* was highly susceptible to larval herbivory whereas all the other tested genotypes were equivalent in their resistance in terms of foliage consumption.

My study indicated that females reared on *S. alba* developed into heavier pupae and produced more silk than on all other host plant genotypes tested; evidently heavier pupae will require more silk for better attachment to the substrate. Pupal weight is often considered a good indicator of offspring fitness as heavier pupae are known to produce larger and more fecund adults than their smaller counterparts (Barah and Sengupta, 1991; Armbruster and Hutchinson, 2002), but this has never been reported previously for *P. xylostella.* This is the first evidence to suggest that different plant genotypes affect female pupal weights differently and, in turn, can influence adult bodyweight, longevity (without food) and forewing area. Although oviposition differed significantly among females raised on various host plants, lack of correlation between pupal weight and number of eggs laid indicated that parameters like oviposition depend, at least in part, on other environmental factors in addition to host plants. However, food plants affected the oviposition site selection by new generation adults. For instance, females reared on *B. napus* cv. Conquest exhibited less discrimination between substrates and deposited most eggs off the leaf disc compared with females raised on other tested host plants.

Among genotypes evaluated, the greatest levels of overall antibiotic resistance were observed in *B. napus* cv. Liberty and *B. oleracea.* For instance, females reared on *B. napus* cv. Liberty had lighter pupal weights, less silk, lighter adult bodyweights and reduced longevities (without adult food) compared with other plant genotypes tested. Similarly, *B. oleracea* had higher antibiotic resistance against *P. xylostella* females than *S. alba* as indicated by slower larval and pupal development, lighter pupal weight, less silk, lower bodyweight, shorter forewings, reduced longevity without food, and reduced reproduction of new generation adults. Such antibiotic effects could cause reductions in fitness of *P. xylostella*; for example, prolonged developmental time could increase the exposure of the insect to its natural enemies. Reduced longevity when food is limiting could impair reproductive success if females are not in the habitats of favorable host plants. Reduced wing area could also affect fitness. *Plutella xylostella* adults are highly migratory (Talekar and Shelton, 1993; Chapman et al., 2002) and moths with larger wings fly more actively than those with smaller wings (Muhamad et al., 1994; Begum et al., 1996). Female moths reared on *B. napus* cv. Liberty and *B. oleracea* had the smallest forewings suggesting that they would have reduced dispersal capability than those fed on plants such as *B. napus* cv. Conquest, *B. rapa*, *B. carinata* and *S. alba.*

3.4.3 Root Mass Development in Response to Insect Herbivory

The present study suggests that when under stress from *P. xylostella* herbivory, host plants compensate by increasing their root masses. For example, *B. napus* cv. Q2, *B. napus* cv. Conquest, *B. rapa*, *B. carinata* and *S. alba* plants infested with *P. xylostella* had significantly more robust root systems than their uninfested counterparts. Greater root mass development in infested plants could enable more efficient extraction from the soil of elements like nitrogen and sulfur that are required in the defense responses by plants. Plants can also increase their net aboveground productivity when infested (Belsky, 1986 and references therein). However, not all Brassicaceae tested had similar compensatory capabilities: the root masses of *B. napus* cv. Liberty, *B. juncea* and *B. oleracea* did not differ significantly between infested and uninfested plants (Figure 3-III).

Table 3-I. Mean percent survival (± S.E.) of *Plutella xylostella* from neonate to pupa and pupa to adult on whole plants (n = 10) of different Brassicaceae.

Host Plant Genotype	Survival (%)	
	Neonate to Pupa	Pupa to Adult
Brassica napus cv. Q2	72.00 ^{a}	97.5 ^{a}
	(3.74)	(2.50)
Brassica napus cv. Liberty	80.00 ^{a}	96.00 ^{a}
	(8.37)	(2.45)
Brassica napus cv. Conquest	80.00 ^{a}	94.28 ^{a}
	(4.47)	(3.50)
Brassica juncea	86.00 ^{a}	92.70 ^{a}
	(4.00)	(4.64)
Brassica rapa	86.00 ^{a}	95.78 ^{a}
	(6.78)	(2.59)
Brassica carinata	78.00 ^{a}	90.92 ^{a}
	(7.35)	(3.97)
Brassica oleracea	69.00 ^{a}	91.58 ^{a}
	(6.78)	(3.55)
Sinapis alba	82.00 ^{a}	100.00 ^{a}
	(4.90)	(0.00)

Means in a column followed by the same letter do not differ significantly (*P* = 0.05) using analysis of variance and Tukey's studentized range test

Table 3-II. Mean (± S.E.) developmental time of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when reared on leaf tissue of various Brassicaceae.

	Developmental time (days)					
Host Plant Genotype	Larva to Pre-pupa		Pre-pupa to Pupa		Pupa to adult	
	Female	Male	Female	Male	Female	Male
Brassica napus	6.40^{a}	5.60^{dc}	1.00^{a}	0.90^{ab}	4.30^{b}	5.30^{cd}
cv. Q2	(0.15)	(0.18)	(0.00)	(0.05)	(0.11)	(0.15)
Brassica napus	5.60^{bc}	4.10^{e}	0.85^{a}	1.00^{a}	4.70^{ab}	5.70^{abc}
cv. Liberty	(0.11)	(0.12)	(0.05)	(0.00)	(0.11)	(0.18)
Brassica napus	5.40^{bc}	5.80^{c}	0.95^{a}	0.80^{ab}	4.30^{b}	5.10^{d}
cv. Conquest	(0.18)	(0.14)	(0.03)	(0.06)	(0.11)	(0.07)
Brassica juncea	5.00^{c}	6.10^{bc}	0.80^{a}	0.75^{b}	4.90^{a}	5.60^{bcd}
	(0.18)	(0.12)	(0.06)	(0.06)	(0.19)	(0.11)
Brassica rapa	6.50^{a}	6.50^{ab}	0.85^{a}	0.85^{ab}	4.70^{ab}	6.20^{a}
	(0.15)	(0.15)	(0.10)	(0.10)	(0.15)	(0.17)
Brassica	6.90^{a}	6.80^{a}	0.95^{a}	0.90^{ab}	4.40^{ab}	6.10^{ab}
carinata	(0.19)	(0.22)	(0.03)	(0.05)	(0.15)	(0.12)
Brassica	6.80^{a}	6.10^{bc}	1.05^{a}	0.95^{ab}	4.90^{a}	6.00^{ab}
oleracea	(0.14)	(0.16)	(0.36)	(0.03)	(0.12)	(0.10)
Sinapis alba	5.70^{b}	5.10^{d}	0.90^{a}	0.90^{ab}	4.20^{b}	5.30^{cd}
	(0.11)	(0.16)	(0.05)	(0.05)	(0.09)	(0.15)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 3-III. Mean (± S.E.) foliage consumption, pupal weight, and silk weight of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when reared on leaf tissue of various Brassicaceae.

Host Plant Genotype	Foliage consumed (cm^2)		Pupal weight (mg)		Silk weight (mg)	
	Female	Male	Female	Male	Female	Male
Brassica napus	3.02 ^{b}	2.13 ^{ab}	7.28 ^{dc}	5.52 ^{a}	0.26 ^{b}	0.21 ^{ab}
cv. Q2	(0.13)	(0.16)	(0.06)	(0.06)	(0.01)	(0.00)
Brassica napus	2.96 ^{b}	1.95 ^{b}	6.29 ^{e}	5.83 ^{a}	0.22 ^{b}	0.21 ^{ab}
cv. Liberty	(0.16)	(0.01)	(0.18)	(0.08)	(0.01)	(0.01)
Brassica napus	2.65 ^{b}	2.45 ^{ab}	7.64 ^{bc}	5.84 ^{a}	0.26 ^{b}	0.24 ^{a}
cv. Conquest	(0.19)	(0.10)	(0.14)	(0.09)	(0.01)	(0.01)
Brassica juncea	2.54 ^{b}	2.24 ^{ab}	7.71 ^{bc}	5.72 ^{a}	0.24 ^{b}	0.20 ^{bc}
	(0.16)	(0.18)	(0.07)	(0.03)	(0.01)	(0.00)
Brassica rapa	3.75 ^{a}	2.56 ^{a}	8.04 ^{ab}	5.89 ^{a}	0.25 ^{b}	0.21 ^{ab}
	(0.24)	(0.14)	(0.15)	(0.17)	(0.01)	(0.01)
Brassica	2.62 ^{b}	2.08 ^{ab}	7.60 ^{bc}	5.63 ^{a}	0.22 ^{b}	0.17 ^{c}
carinata	(0.03)	(0.09)	(0.16)	(0.16)	(0.01)	(0.01)
Brassica	3.03 ^{b}	2.20 ^{ab}	6.93 ^{d}	4.62 ^{b}	0.24 ^{b}	0.22 ^{ab}
oleracea	(0.09)	(0.07)	(0.09)	(0.12)	(0.01)	(0.00)
Sinapis alba	2.37 ^{b}	1.97 ^{b}	8.57 ^{a}	5.79 ^{a}	0.38 ^{a}	0.23 ^{ab}
	(0.11)	(0.16)	(0.14)	(0.11)	(0.04)	(0.01)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 3-IV. Mean (± S.E.) adult body weight, forewing area and longevity of female (n = 20) and male (n = 20) *Plutella xylostella* when reared as larvae on different Brassicaceae.

Host Plant Genotype	Adult bodyweight (mg)		Forewing area (cm^2)		Longevity (days)		
					No-food		Food
	Female	Male	Female	Male	Female	Male	Female
Brassica napus	2.58^{b}	1.54^{ab}	0.15^{bc}	0.13^{bc}	8.20^{b}	5.40^{ab}	23.20^{a}
cv. Q2	(0.06)	(0.05)	(0.00)	(0.00)	(0.14)	(0.11)	(0.86)
Brassica napus	2.09^{c}	1.65^{a}	0.14^{c}	0.12^{c}	6.50^{d}	5.00^{bc}	24.80^{a}
cv. Liberty	(0.05)	(0.04)	(0.00)	(0.00)	(0.11)	(0.10)	(1.62)
Brassica napus	2.50^{bc}	1.24^{c}	0.17^{a}	0.15^{a}	7.30^{c}	5.80^{a}	25.20^{a}
cv. Conquest	(0.07)	(0.01)	(0.00)	(0.00)	(0.18)	(0.14)	(1.11)
Brassica juncea	2.35^{bc}	1.11^{c}	0.15^{bc}	0.13^{bc}	8.10^{b}	5.50^{ab}	24.40^{a}
	(0.07)	(0.02)	(0.00)	(0.00)	(0.19)	(0.11)	(1.33)
Brassica rapa	2.43^{bc}	1.28^{bc}	0.16^{ab}	0.14^{ab}	8.20^{b}	5.10^{bc}	24.60^{a}
	(0.11)	(0.07)	(0.00)	(0.00)	(0.14)	(0.24)	(2.38)
Brassica	2.41^{bc}	1.13^{c}	0.16^{ab}	0.14^{ab}	6.90^{dc}	5.40^{ab}	25.00^{a}
carinata	(0.16)	(0.09)	(0.00)	(0.00)	(0.12)	(0.15)	(1.18)
Brassica	2.35^{bc}	1.15^{c}	0.14^{c}	0.15^{a}	6.70^{dc}	4.50^{c}	22.6^{a}
oleracea	(0.10)	(0.10)	(0.00)	(0.00)	(0.29)	(0.11)	(1.21)
Sinapis alba	3.55^{a}	1.15^{c}	0.16^{ab}	0.13^{bc}	9.50^{a}	5.30^{ab}	24.00^{a}
	(0.04)	(0.04)	(0.00)	(0.00)	(0.15)	(0.15)	(0.84)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 3-V. Pair-wise comparison (*t*- values) between female (n = 20) and male (n = 20) *Plutella xylostella* for some key life history parameters when reared on the same host plant genotype.

Biological Parameters	*B. napus* Q2	*B. napus* Liberty	*B. napus* Conquest	*B. juncea*	*B. rapa*	*B. carinata*	*B. oleracea*	*S. alba*
Larval dev. time	3.36**	8.89***	-1.74 ^{ns}	- 5.08***	0.00 ^{ns}	0.34 ^{ns}	3.31**	3.13**
Pre-pupal dev. time	2.18*	- 2.85**	2.28*	0.62 ^{ns}	0.00 ^{ns}	0.87 ^{ns}	1.14 ^{ns}	0.00 ^{ns}
Pupal dev. time	- 5.54***	- 4.81***	- 6.37***	- 3.16**	- 6.64***	- 8.67***	- 6.85***	- 6.35***
Herbivory (per larva)	4.35***	6.17***	0.98 ^{ns}	1.27 ^{ns}	4.30***	5.69***	7.07***	2.05 ^{ns}
Pupal weight	20.13***	2.37*	10.91***	26.09***	9.23***	8.88***	15.89***	15.23***
Silk weight	5.01***	1.19 ^{ns}	1.70 ^{ns}	5.60***	4.24***	3.69***	3.52**	4.05***
Adult body weight	14.06***	6.56***	12.86***	16.19***	8.92***	7.01***	8.37**	41.68***
Forewing area	3.28**	2.29*	5.03***	4.30***	6.07***	3.23**	- 0.58 ^{ns}	4.47***
Adult longevity without food	15.76*	9.75***	6.64***	11.69***	11.22***	7.65***	7.03***	19.74***

^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

Table 3-VI. Mean (± S.E.) eggs deposited per female (n = 10) of new generation adults reared as larvae on various brassicaceous species and cultivars.

Host Plant Genotype	Oviposition over an 8-d period		
	Eggs on the leaf disc	Eggs off the leaf disc	Total number of eggs
Brassica napus	161.30 ^{c}	68.20 ^{dc}	229.50 ^{c}
cv. Q2	(8.26)	(9.30)	(7.40)
Brassica napus	201.50 ^{b}	74.20 ^{c}	275.70 ^{b}
cv. Liberty	(6.73)	(3.52)	(8.45)
Brassica napus	310.20 ^{a}	126.60 ^{a}	436.80 ^{a}
cv. Conquest	(3.80)	(4.29)	(6.69)
Brassica juncea	302.20 ^{a}	99.40 ^{b}	401.60 ^{a}
	(8.47)	(3.36)	(7.51)
Brassica rapa	151.50 ^{c}	60.00 ^{dc}	211.50 ^{c}
	(5.97)	(4.45)	(8.30)
Brassica	159.70 ^{c}	54.40 ^{d}	214.10 ^{c}
carinata	(6.50)	(3.58)	(7.56)
Brassica	112.20 ^{d}	68.20 ^{dc}	180.40 ^{d}
oleracea	(5.33)	(3.51)	(6.86)
Sinapis alba	195.90 ^{b}	94.00 ^{b}	289.90 ^{b}
	(5.49)	(6.40)	(2.29)

Means in a column followed by the same letter do not differ significantly (*P* = 0.05) using analysis of variance and Tukey's studentized range test

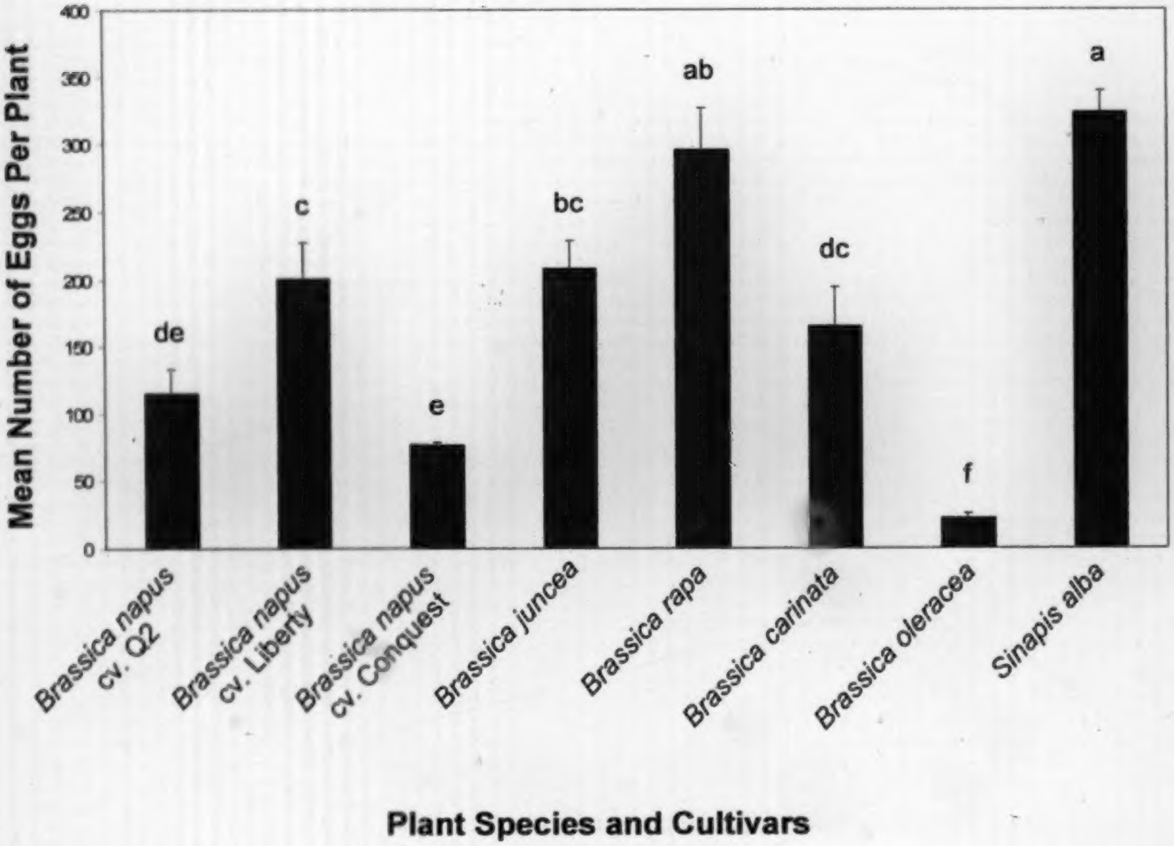

Figure 3-I. Ovipositional preference by *Plutella xylostella* in free-choice tests (n = 5). Means and standard errors are presented; means with different lowercase letters are significantly different from each other (ANOVA and Tukey's mean separation, *P* = 0.05).

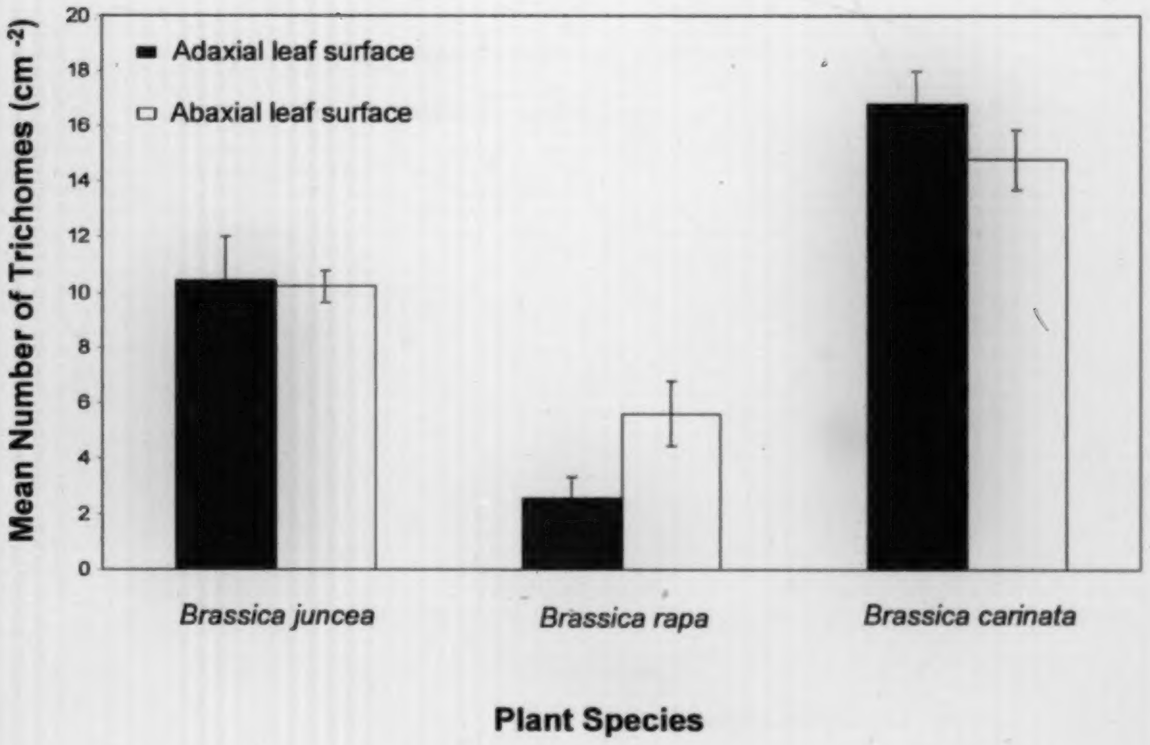

Figure 3-II. Trichome density on adaxial and abaxial surface of leaf discs of *Brassica rapa*, *B. juncea* and *B. carinata*. Means and standard errors are presented. *Brassica napus* cv. Q2, *B. napus* cv. Liberty, *B. napus* cv. Conquest, *B. oleracea* and *Sinapis alba* are not shown in the figure as they lacked trichomes on the adaxial and abaxial leaf surfaces.

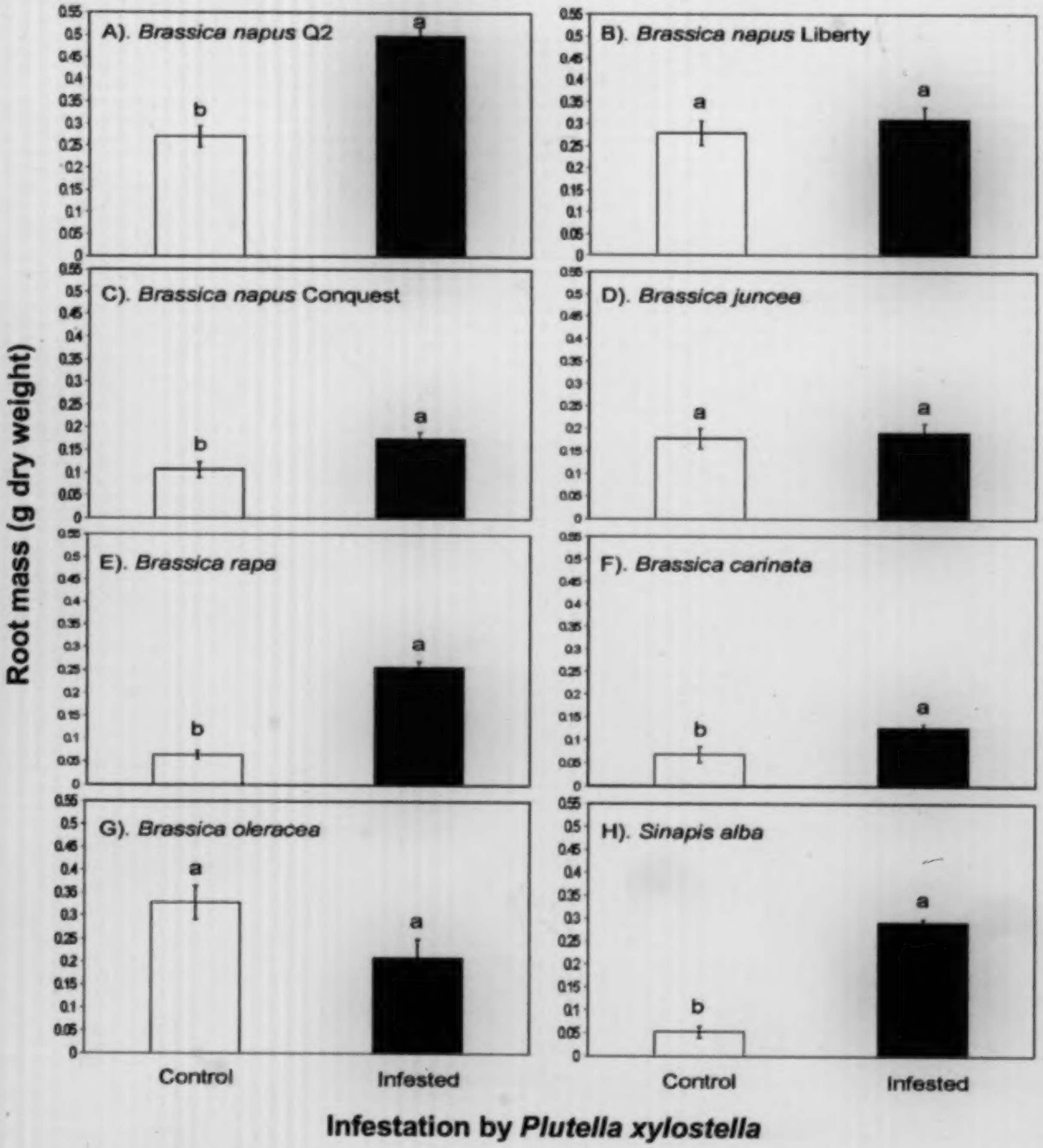

Figure 3-III. Root mass development of various Brassicaceae species and cultivars when infested (n = 5) and noninfested (n = 5) by *Plutella xylostella* larvae. Means and standard errors are presented; in each graph, means with different lowercase letters are significantly different from each other at *P* = 0.05.

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Chapter 4:

Performance of the Specialist Herbivore *Plutella xylostella* on Brassicaceous and Non-brassicaceous Species

4.1 Introduction

Many herbivorous insects can expand their host ranges by incorporating new food plants into their diets. Cases of host range expansion by herbivores include novel plants from the same genus or family (Hsiao, 1978; Tabashnik, 1983; Feder et al., 1990; Carroll and Boyd, 1992; Singer et al., 1993; Fraser and Lawton, 1994; Camara 1997), and from a different family (Phillips and Barnes, 1975; Gould, 1979; Bowers et al., 1992; Löhr and Gathu, 2002). For a novel plant to be incorporated into the diet, the insect herbivore must recognize that plant as food and be able to survive on it. There are several components to this process, including the ability of females to locate the host plant and to accept it as an oviposition site, larval acceptance of the plant for feeding, and the capability of larvae to incur sufficient growth and survival on the new host (Rausher, 1982; Thomas et al., 1987; Thompson, 1988; Bowers et al., 1992). In turn, the use of a new host plant may increase food availability; however, alteration of food quality may affect offspring fitness, various morphometric variations and physiological costs can arise from using the new host plant, and there can be changes in the vulnerability of the opportunist herbivore to its natural enemies (Price et al., 1980; Scriber and Slansky, 1981; Tabashnik, 1983; Bernays and Graham, 1988; Weis and Berenbaum, 1989; Bowers et al., 1992; Bernays and Chapman, 1994; Begum et al., 1996; Camara, 1997).

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered a specialist on Brassicaceae (Talekar and Shelton, 1993; Muhamad et al., 1994; Begum et al., 1996; Idris and Grafius, 1996; Sarfraz et al., 2006). Brassicaceae comprise a diverse group of 380 genera and over 3000 species of cultivated and wild plants (Heywood, 1993) that are characterized by secondary plant compounds, the glucosinolates (Kjaer, 1974; Mithen, 1992). *Plutella xylostella* relies on some of the glucosinolates for host location, oviposition and feeding stimulation (Thorsteinson, 1953; Gupta and Thorsteinson, 1960b; Marazzi et al., 2004). For instance, host plant cues stimulated the onset of its reproductive activities (Pittendrigh and Pivnick, 1993), but the presence of non-host plants did not produce this effect (Hillyer and Thorsteinson, 1969; Renwick and Radke, 1990).

Brassicaceae and close allies such as Capparaceae and Troppaeolaceae commonly occur in many parts of the world. Canola, *Brassica napus* L. (Brassicaceae), is a widely cultivated oilseed crop in North America, Europe, Asia and Oceania. Throughout the world, vast areas are grown in canola monocultures; for example in western Canada its production extends over more than 5 million ha annually (Canola Council of Canada, 2006). Flixweed, *Descurainia sophia* (L.) Webb ex Prantl (Brassicaceae) is a winter annual or biennial and in temperate North America it is one of the first weeds to appear in the spring. It is native to Europe but is now common throughout North America (Morishita, 1991; Mitich, 1996). The spider flower, *Cleome hassleriana* Chodat (Capparaceae), is a common annual ornamental herb in North America (Foster, 2001) and it readily escapes gardens to invade roadsides and the shores of rivers and lakes (Anonymous, 2006). The garden nasturtium, *Tropaeolum majus* L. (Tropaeolaceae), originated in South America but is now grown worldwide as an ornamental plant (Stephens, 2003) and often occurs as a weed on roadsides and riverbanks. Glucocapparin (aliphatic allylic glucosinolate) and glucotropaeolin (aromatic glucosinolate) predominate in *C. hassleriana* and *T. majus*, respectively (Kjaer, 1974; Renwick and Lopez, 1999). Despite their close chemical relationship with Brassicaceae by containing glucosinolates, these non-brassicaceous plants have never been reported to be infested with *P. xylostella*.

Cleome hassleriana and *T. majus* can occur sympatrically with wild and volunteer brassicaceous plants, and so could potentially harbor *P. xylostella* populations, perhaps providing bridge hosts until crop plants are available. Therefore, in this study I tested ovipositional preferences of *P. xylostella* in a free-choice situation using two brassicaceous (*B. napus* and *D. sophia*) and two non-brassicaceous species (*C. hassleriana* and *T. majus*). No-choice tests were conducted to investigate several developmental parameters of *P. xylostella* on all four plant species. The study was designed to investigate a grouping of taxonomically distinct but chemically-related plants to provide insight into insect-plant interactions using an oligophagous herbivore.

4.2 Materials and Methods

4.2.1 Insects and Plants

The laboratory colony of *P. xylostella* was maintained on potted *B. napus* plants at 22±0.5°C with 16h L: 8h D under growth chamber conditions. Moths collected from different fields in Alberta, Canada were added to the culture every summer to maintain genetic diversity.

Brassica napus 'Q2', *D. sophia*, *C. hassleriana* 'Cherry Queen' and *T. majus* 'Golden Gleam' were grown under greenhouse conditions. Seeds of *B. napus* were kindly provided by Drs. Dosdall and Keddie whereas seeds of *D. sophia* were collected from Edmonton and Lethbridge areas. Seeds of *C. hassleriana* and *T. majus* were provided by Apache Seeds Ltd., Edmonton. Plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium fertilized with 20: 20: 20 (nitrogen: phosphorous: potassium) at 0.5 g pot^{-1} when plants were two to four weeks old. Depending on the growth habits of tested plant species, seeding dates were adjusted accordingly. Five- to seven-week-old plants were used for all experiments; i.e., fast-growing species (*B. napus* and *T. majus*) were used when plants were five weeks old while slow-growing species (*D. sophia* and *C. hassleriana*) were six to seven weeks old.

4.2.2 Intact Plant Study

4.2.2.1 *Plutella xylostella* Oviposition Preference

Ovipositional preference experiments were conducted with *P. xylostella* in a free-choice situation in ten screened cages (120 x 120 x 120 cm) using a randomized complete block design and each cage was considered a block. One plant from each species was placed in a random position within each cage and a total of 40 plants were used in the experiment (i.e., four plants per cage). Eight one-day-old adults (two moths per plant) were released in 1:1 (m: f) sex ratio in the middle of each cage and provided with a sterile 10% honey solution for adult feeding. Eggs on each plant were counted 2 d following moth release.

4.2.2.2 *Plutella xylostella* Survival

Survival from neonate to pupa was assessed in screened cages (40 x 40 x 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 40 cages with 10 plants from each species infested with first-instar larvae (at 10 larvae per plant). Plants were observed every 48 h and the numbers of surviving individuals were recorded. Pupae were harvested, weighed and kept individually in transparent plastic cups until adult emergence.

4.2.3 Excised Leaf Tissue Study

4.2.3.1 Pre-imaginal Developmental Parameters

This study was carried out in controlled environmental conditions (22±0.5°C with 16h L: 8h D) following the protocol described by Sarfraz et al. (2007). Excised leaves were placed on moist filter papers in 170 ml plastic containers with perforated lids to ensure ventilation and to avoid condensation. For each plant species, 100 to 200 second-instar larvae (≤ 1 day old) taken from the laboratory colony were introduced into individual plastic containers; a total of 600 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Developmental times from second-instar larva to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were weighed using a Sartorius Supermicro® balance (Sartorius Inc., Edgewood, NY, USA) as described by Sarfraz et al. (2007).

To quantify levels of larval feeding, all leaves damaged by *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA). Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory. Leaves of *D. sophia* were too small to be scanned. In this case, I had two sets of cups containing leaves: the first set received larvae whereas second set served as uninfested controls. Both infested and uninfested leaves were weighed daily and the amount of foliage removed due to larval herbivory was quantified.

4.2.3.2 Imaginal Parameters

Twenty females and 20 males reared from each plant taxon were used to determine their longevities without food. Moths were weighed within 24 h of their death. Forewings were carefully removed, glued onto a paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA), and their areas were measured using Image J.

Ten male/female pairs of moths of almost the same age (≤ 1 day old) reared from each plant taxon were released in individual plastic containers (i.e., one pair per cup) containing *B. napus* cv. Q2 leaf discs (area = 52.8 cm^2) placed on moist filter papers. A total of 40 cups were used following a completely randomized design and this experiment was conducted under controlled environmental conditions (22±0.5°C with 16h L: 8h D). Insects were allowed to oviposit over an 8-d period and eggs found on leaf discs, filter papers, and containers were recorded daily. Every day, the leaf discs were taken from undamaged plants to avoid any induced effects on oviposition by *P. xylostella*. Every 24 h, moths were transferred to new containers and provided with fresh leaf discs and food (sterile 10% honey solution) as described by Sarfraz et al. (2007).

4.2.4 Root Mass Development in Response to Insect Herbivory

At the end of the experiment investigating *P. xylostella* survival on whole plants, infested and uninfested plants were uprooted; their roots were carefully washed, air-dried at room temperature and weighed to determine the effects of aboveground herbivory on root mass (mg dry weight basis) development.

4.2.5 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used as necessary to achieve normality and homoscedasticity before analysis (Steel et al., 1997), but untransformed means are presented. Analysis of variance (ANOVA) (PROC GLM) for a randomized complete block design was performed for oviposition choice experiments with cage as a blocking factor. ANOVA for a completely randomized design was performed as necessary to test the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). Data on larval herbivory for *D. sophia* were not included in the ANOVA owing to the use of different measurement units from other tested plant species. Correlation coefficients were determined between pupal weight and silk weight, pupal weight and adult weight, pupal weight and longevity without food, pupal weight and forewing area, adult weight and longevity, and pupal weight and oviposition. T-tests (PROC TTEST) were performed for pair-wise comparisons between female and male specimens for their development time, larval herbivory, pupal weight, silk weight, adult body weight, forewing area and longevity (without food) when they were reared on various plant species.

4.3 Results

4.3.1 Intact Plant Study

4.3.1.1 *Plutella xylostella* Oviposition Preference

Plant species had significant effects on *P. xylostella* oviposition preference ($F_{3,27} = 133.57$; $P < 0.0001$) (Figure 4-I). Oviposition on *D. sophia* and *C. hassleriana* were similar, but numbers of eggs deposited on *B. napus* exceeded other plant species. Females laid 2.2-, 2.3-, and 15.1-fold more eggs on *B. napus* than on *D. sophia*, *C. hassleriana* and *T. majus* respectively (Figure 4-I).

4.3.1.2 *Plutella xylostella* Survival

Plant species had significant effects on survival of *P. xylostella* from neonate to pupa ($F_{3,27} = 7.81$; $P = 0.0037$), but not from pupa to adult ($F_{3,27} = 0.11$; $P = 0.9536$) (Table 4-I). Percent survival from neonate to pupa on *B. napus* was significantly greater than on *D. sophia* and *T. majus*, but survival did not differ significantly between *B. napus* and *C. hassleriana*; the lowest survival occurred on *D. sophia* (Table 4-I).

4.3.2 Excised Leaf Tissue Study

4.3.2.1 Pre-imaginal and Imaginal Parameters

Plant species significantly affected larval and pupal development times of females ($F_{3,57} = 5.12$; $P = 0.0033$ and $F_{3,57} = 15.95$; $P < 0.0001$ respectively) and males ($F_{3,57} = 4.82$; $P = 0.0046$ and $F_{3,57} = 13.48$; $P < 0.0001$ respectively) (Table 4-II). Pre-pupal development time was significantly affected for females ($F_{3,57} = 4.37$; $P = 0.0077$), but not for males ($F_{3,57} = 0.51$; $P = 0.6746$) (Table 4-II). Plant species had significant effects on foliage consumption by individual female and male larvae (*D. sophia* not included in analysis) ($F_{3,57} = 17.06$; $P < 0.0001$ and $F_{3,57} = 3.30$; $P = 0.0478$ respectively) (Figure 4-II). Pupal weight and silk weight differed significantly for females ($F_{3,57} = 27.74$; $P < 0.0001$ and $F_{3,57} = 14.53$; $P < 0.0001$ respectively) and males ($F_{3,57} = 6.82$; $P = 0.0005$ and $F_{3,57} = 7.74$; $P = 0.0002$ respectively) when reared on various plant species (Table 4-II).

Female larval development was faster on *B. napus* than on *D. sophia* whereas male larvae developed faster on *B. napus* than on *T. majus* (Table 4-II). Female pupae developed fastest on *B. napus* and *D. sophia* whereas male pupal development was fastest on *C. hassleriana*. Both female and male larvae consumed similar leaf areas of *B. napus* and *C. hassleriana* but least foliage consumption occurred on *T. majus* (Figure 4-II). On *D. sophia*, female and male larvae consumed 0.08 g and 0.05 g of foliage, respectively. Female pupae were heaviest on *B. napus* and lightest on *D. sophia*; male pupae were significantly heavier on *B. napus* than on non-Brassicaceae (Table 4-II). Female specimens reared on *D. sophia* produced the least amount of silk. Females and males reared on *B. napus* and *T. majus* produced statistically similar amounts of silk. For female and male specimens reared on various plant species, a significant correlation was found between pupal weight and silk weight ($r = 0.71$; $P < 0.0001$ and $r = 0.62$; $P < 0.0001$ respectively).

Plant species significantly affected forewing area and longevity (without food) of females ($F_{3,57} = 23.40$; $P < 0.0001$ and $F_{3,57} = 20.84$; $P < 0.0001$ respectively) and males ($F_{3,57} = 15.33$; $P < 0.0001$ and $F_{3,57} = 11.79$; $P < 0.0001$ respectively) (Table 4-III). Adult body weight was significantly affected by plant species for females ($F_{3,57} = 22.24$; $P < 0.0001$), but not for males ($F_{3,57} = 1.78$; $P = 0.1610$) (Table 4-III).

Heaviest females were produced on *B. napus*; lightest females developed on *T. majus* and *D. sophia* (Table 4-III). Body masses for males reared on all the tested plant species did not differ significantly from each other. Female moths reared on *D. sophia* had the smallest forewings whereas forewing areas were similar for females on *B. napus*, *C. hassleriana* and *T. majus*. Male moths reared on *B. napus* and *C. hassleriana* had the largest forewings. Females reared on *B. napus* and *C. hassleriana* lived longer in the absence of food than those reared on either *D. sophia* or *T. majus*. Males reared on *T. majus* lived for the shortest time without food (Table 4-III). For females, a significant correlation was found between pupal weight and adult body weight ($r = 0.73$; $P < 0.0001$), pupal weight and forewing area ($r = 0.46$; $P < 0.0001$), and pupal weight and longevity (without food) ($r = 0.48$; $P < 0.0001$). For males, a significant correlation was observed between pupal weight and adult weight ($r = 0.78$; $P < 0.0001$), pupal weight and forewing area ($r = 0.40$; $P = 0.0002$), and pupal weight and longevity (without food) ($r = 0.36$; $P = 0.0011$).

Pupal weights and oviposition were positively correlated for females reared on all tested plant species ($r = 0.48$; $P < 0.0001$). Females raised on the brassicaceous and non-brassicaceous plants deposited significantly different numbers of total eggs over the 8-day oviposition period ($F_{3,27} = 41.85$; $P < 0.0001$). Plant species significantly affected oviposition on days 1 ($F_{3,27} = 37.10$; $P < 0.0001$), 2 ($F_{3,27} = 13.78$; $P < 0.0001$), 3 ($F_{3,27} = 21.57$; $P < 0.0001$), 4 ($F_{3,27} = 6.88$; $P = 0.0014$), 5 ($F_{3,27} = 6.64$; $P = 0.0017$), and 7 ($F_{3,27} = 5.06$; $P = 0.0066$). Oviposition did not differ on days 6 ($F_{3,27} = 1.63$; $P = 0.2066$), and 8 ($F_{3,27} = 1.74$; $P = 0.1832$) among females raised on the plant species tested. Females reared on *D. sophia* deposited the fewest total eggs over the 8-day period whereas total oviposition did not differ for females raised on all other tested plant species. On day 1, females reared on *T. majus* and *B. napus* laid the most eggs whereas moths from *D. sophia* deposited fewest eggs (Figure 4-III). On days 2, 3 and 4, females raised on *B. napus*, *C. hassleriana* and *T. majus* deposited similar numbers of eggs but significantly more than those reared on *D. sophia*. Females from *D. sophia* and *C. hassleriana* had similar oviposition on days 5 and 7. Oviposition did not differ significantly on days 6 and 8 for females raised on the tested plant species (Figure 4-III).

Female and male specimens exhibited significant differences in various life history traits when reared as larvae on leaf tissue of the same plant species (Table 4-IV). For instance, male pupal development was significantly slower than their female counterparts on all plant species except *C. hassleriana*. Compared with males, females consumed more foliage of all the tested plant species during their larval development. Female pupae were heavier than their male counterparts on all four species. Body weights of female and male moths were similar when larvae were reared on *D. sophia* whereas female moths were significantly heavier than males when reared on *B. napus*, *C. hassleriana* and *T. majus*. Males had smaller forewings than females when reared as larvae on the tested plant species except *D. sophia*. Females lived longer than their male counterparts in the absence of food when larvae were reared on any tested plant species (Table 4-IV).

4.3.3 Root Mass Development in Response to Insect Herbivory

Pair-wise comparisons for each species indicated that infested *B. napus* plants had significantly more robust root systems than their uninfested counterparts (t = 14.71; $P <$ 0.0001) (Figure 4-IV). The root masses of infested *C. hassleriana* and *T. majus* plants were significantly less than their uninfested counterparts (t = - 8.87; $P < 0.0001$ and t = - 8.06; $P < 0.0001$ respectively). However, the root masses of *D. sophia* did not differ significantly between infested and uninfested plants (t = - 0.05; P = 0.9581) (Figure 4-IV).

4.4 Discussion

4.4.1 Oviposition Preference

This study demonstrates that females accepted *C. hassleriana* as a host even in the presence of more favorable plants such as *B. napus*. *Cleome hassleriana* could potentially serve as an efficient bridge host for *P. xylostella* populations when no Brassicaceae are available in the habitat, but to my knowledge, no published field evidence exists to support or refute this hypothesis. *Tropaeolum majus* received the fewest eggs among the plant species tested. In an earlier study, *T. majus* extracts provided only a limited enhancement effect for *P. xylostella* oviposition when applied to Sieva bean plants, *Phaseolus vulgaris* L. (Fabaceae) (Renwick and Radke, 1990). It is perhaps not surprising, therefore, that *P. xylostella* infestations have never been reported on this species under field conditions.

4.4.2 Pre-imaginal and Imaginal Parameters

Larval herbivory is a good indicator of food plant suitability, and my study demonstrated that *P. xylostella* larvae can accept *C. hassleriana* and *T. majus* as food plants and perform reasonably well on these non-brassicaceous species. *Plutella xylostella* larvae need specific stimulants to accept a plant and initiate feeding (Gupta and Thorsteinson, 1960a; van Loon et al., 2002) and may even starve to death on unsuitable plants (Sarfraz et al., 2006). For instance, larvae of *P. xylostella* did not feed on non-cruciferous plants such as *Dahlia* sp., *Gynura* sp., *Chrysanthemum* sp., *Cucumis* sp., *Euphorbia poinsettiana* Buist., *E. splendens* Bojer ex Hook, *Abutilan* sp., *Maranta* sp., *Pepromia* sp., *Rhamnus* sp., *Clematis* sp. and *Rosa* sp., but they readily accepted leaf discs of these plants when discs were treated with sinigrin, a glucosinolate compound (Gupta and Thorsteinson, 1960a). Brassicaceae are heavily dominated by long-chain glucosinolates. By contrast, the short-chain alanine-derived methylglucosinolates predominate in Capparaceae, but have not been detected in Brassicaceae (Kjaer, 1974). A similar amount of herbivory on *B. napus* and *C. hassleriana* suggests that larvae probably did not discriminate between long- and short-chain glucosinolates, and that both plant species are suitable for *P. xylostella* larval development.

Female larval developmental time was similar on Brassicaceae and non-Brassicaceae, but pupal developmental time for larvae reared on *C. hassleriana* and *T. majus* was significantly longer than on bassicaceous plants. Prolonged developmental time would be disadvantageous to *P. xylostella* survival under field conditions mainly because of increased potential threats from natural enemies (Williams, 1999). Moreover, insects in the pupal stage are particularly vulnerable to predation and parasitism as this stage has the highest biomass-to-volume ratio and pupae have limited or no movement capability to escape attacks from natural enemies (White, 1987; Barker et al., 2006). The longer duration of the pupal stage on the two non-brassicaceous hosts could therefore predispose individuals to greater mortality from natural enemies in the event of such host expansion by *P. xylostella*.

Plant species affected pupal weights, and this in turn influenced key imaginal parameters including body weight, forewing area, longevity and oviposition. Similar to the present study, forewing area was correlated with pupal weight in *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae) (Evenden et al., 2006) and in *P. xylostella* (Sarfraz et al., 2007). Although I did not measure the wing areas of females used in oviposition tests, on the basis of positive correlation between pupal weight and oviposition, and pupal weight and forewing area in no-food experiments, I suspect that *P. xylostella* with larger forewings were more fecund than their smaller counterparts. Similar findings have been reported for *C. conflictana* (Evenden et al., 2006).

Larval food plant significantly affected oviposition of new generation adults. Females reared on *B. napus*, *C. hassleriana* and *T. majus* deposited similar numbers of total eggs over the 8-d period. However, oviposition peaked on day 1 for females raised on *T. majus* whereas most eggs were deposited on day 3 when females were raised on either *B. napus* or *C. hassleriana*. Females from *D. sophia* had the least overall oviposition and most of their eggs were laid on day 5. My results suggest that *D. sophia* was the least suitable host plant among the species tested as indicated by lowest survival, delayed larval development, lighter pupal weight, less silk, lower body mass, smaller forewings, reduced longevity without food, and reduced overall oviposition of new generation adults. The time lag between adult eclosion and peak oviposition coupled with lower body mass when *P. xylostella* are reared on unfavorable host plants such as *D. sophia* may facilitate their migration to more favorable habitats. The latent period could be spent in migration and host plant location, with oviposition to follow after more favorable host plants are found. Alternatively, this strategy may reduce overall fitness of *P. xylostella*; moths would have less oviposition time if they spend more time in migration particularly when they are short-lived in the absence of food.

4.4.3 Root Mass Development in Response to Insect Herbivory

Plant species responded differently to aboveground herbivory in the form of root mass development. The present study indicated that defoliation can increase, decrease, or have no effect on belowground biomass. *Brassica napus* developed a more robust root system when infested, suggesting that these plants may replace tissues lost to herbivory by increased uptake of nutrients from soil. These findings are in accordance with my previous results (Chapter 2). Similarly, turnip plants (*Brassica rapa* L.) produced heavier roots when infested with *P. xylostella* larvae (Taylor and Bardner, 1968). Aboveground herbivory had a non-significant effect on root growth of *D. sophia*. Root mass production in *C. hassleriana* and *T. majus* declined when infested, possibly because a lack of co-evolutionary history with *P. xylostella* had not produced the selection pressure needed for the plants to compensate for herbivory in this way.

There is considerable debate that insect herbivores and their host plants are in a co-evolutionary 'arms race' (Ehrlich and Raven, 1964; Berenbaum, 1983; Berenbaum and Zangerl, 1998; Kareiva, 1999; Zangerl and Berenbaum, 2005). For instance, wild parsnip, *Pastinaca sativa* L. (Apiaceae), is defended against webworms, *Depressaria pastinacella* Duponchel (Oecophoridae), by the presence of furanocoumarins with heritabilities for individual compounds ranging from 0.54 to 0.62; the webworms can metabolize furanocoumarins with heritabilities for cytochrome P450 activity levels ranging from 0.33 to 0.46 (Berenbaum and Zangerl, 1998). Similarly, brassicaceous plants possess the glucosinolate-myrosinase system for their defense but crucifer specialists such as *Pieris rapae* (L.) (Pieridae) and *P. xylostella* have developed various mechanisms to disarm this 'mustard oil bomb' (Ratzka et al., 2002; Wittstock et al., 2004) and can even utilize some of these compounds as token stimuli for their host location. In addition to plant chemistry, if root growth in response to herbivory represents compensatory ability then I suspect that brassicaceous plants might have had better compensation (and even overcompensation) as a result of co-evolution while their non-brassicaceous counterparts could not compensate due to lack of any evolutionary history with *P. xylostella*. Evidently host range shifts by insect herbivores can place substantial pressure on new host plant species to accommodate or compensate for loss of plant tissues, and represents an area in need of future research to uncover underlying mechanisms by which host plants respond to this herbivory.

Table 4-I. Mean (± S.E.) percent survival of *Plutella xylostella* (n = 10) from neonate to pupa and pupa to adult on intact *Brassica napus*, *Descurainia sophia*, *Cleome hassleriana* and *Tropaeolum majus* plants.

Plant Species	% Survival	
	Neonate to pupa	Pupa to adult
Brassica napus	75.00 ^{a}	95.00 ^{a}
	(2.24)	(2.04)
Descurainia sophia	42.00 ^{b}	88.33 ^{a}
	(5.33)	(5.00)
Cleome hassleriana	57.00 ^{ab}	91.33 ^{a}
	(4.48)	(3.82)
Tropaeolum majus	44.00 ^{b}	91.50 ^{a}
	(3.40)	(4.60)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 4-II. Mean (± S.E.) developmental times, pupal weight, and silk weight of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when larvae were reared on leaf tissue of *Brassica napus, Descurainia sophia, Cleome hassleriana* and *Tropaeolum majus*.

Biological parameters		Plant Species			
		Brassica napus	*Descurainia sophia*	*Cleome hassleriana*	*Tropaeolum majus*
Larva to pre-pupa (days)	Female	6.40^{b} (0.15)	7.40^{a} (0.17)	7.00^{ab} (0.24)	6.80^{ab} (0.20)
	Male	5.60^{b} (0.18)	6.15^{ab} (0.22)	6.05^{ab} (0.28)	6.85^{a} (0.21)
Pre-pupa to pupa (days)	Female	1.00^{ab} (0.00)	0.90^{b} (0.05)	0.93^{b} (0.04)	1.15^{a} (0.08)
	Male	0.90^{a} (0.05)	0.88^{a} (0.05)	0.85^{a} (0.05)	0.93^{a} (0.04)
Pupa to adult (days)	Female	4.30^{b} (0.11)	4.50^{b} (0.11)	5.00^{a} (0.13)	5.30^{a} (0.11)
	Male	5.30^{b} (0.15)	5.75^{ab} (0.10)	4.60^{c} (0.24)	5.90^{a} (0.07)
Pupal weight (mg)	Female	7.28^{a} (0.06)	5.78^{c} (0.15)	6.47^{b} (0.12)	6.10^{bc} (0.14)
	Male	5.52^{a} (0.06)	5.18^{ab} (0.13)	4.95^{b} (0.13)	4.87^{b} (0.16)
Silk weight (mg)	Female	0.26^{a} (0.01)	0.16^{c} (0.01)	0.21^{b} (0.00)	0.22^{ab} (0.00)
	Male	0.21^{a} (0.00)	0.16^{c} (0.01)	0.18^{bc} (0.01)	0.19^{ab} (0.01)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 4-III. Mean (± S.E.) adult body weight, forewing area and longevity of *Plutella xylostella* female (n = 20) and male (n = 20) specimens when larvae were reared on leaf tissue of *Brassica napus, Descurainia sophia, Cleome hassleriana* and *Tropaeolum majus*.

		Plant Species			
Biological parameters		*Brassica napus*	*Descurainia sophia*	*Cleome hassleriana*	*Tropaeolum majus*
Adult body weight (mg)	Female	2.58 ^{a}	1.65 ^{c}	2.20 ^{b}	1.58 ^{c}
		(0.06)	(0.11)	(0.14)	(0.04)
	Male	1.54 ^{a}	1.42 ^{a}	1.32 ^{a}	1.29 ^{a}
		(0.05)	(0.09)	(0.13)	(0.06)
Forewing area (cm^2)	Female	0.15 ^{a}	0.11 ^{b}	0.15 ^{a}	0.14 ^{a}
		(0.00)	(0.00)	(0.00)	(0.00)
	Male	0.13 ^{a}	0.12 ^{b}	0.13 ^{a}	0.11 ^{b}
		(0.00)	(0.00)	(0.00)	(0.00)
Longevity without food (days)	Female	8.20 ^{a}	6.20 ^{b}	7.50 ^{a}	6.40 ^{b}
		(0.14)	(0.27)	(0.15)	(0.23)
	Male	5.40 ^{a}	5.00 ^{a}	5.60 ^{a}	4.05 ^{b}
		(0.11)	(0.16)	(0.18)	(0.34)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 4-IV. Pair-wise comparisons (t-values) between female (n = 20) and male (n = 20) *Plutella xylostella* for some key life history parameters when larvae were reared on leaf tissue of *Brassica napus, Descurainia sophia, Cleome hassleriana* and *Tropaeolum majus*.

Biological parameters	Plant Species			
	Brassica napus	*Descurainia sophia*	*Cleome hassleriana*	*Tropaeolum majus*
Development time (larva to pre-pupa)	3.36**	4.50**	2.59**	- 0.17 ^{ns}
Development time (pre-pupa to pupa)	2.18*	0.37 ^{ns}	1.13 ^{ns}	2.46*
Development time (pupa to adult)	- 5.54***	- 8.24***	1.45 ns	- 4.77***
Herbivory (per larva)	4.35***	5.48***	5.98***	3.21**
Pupal weight	20.13***	2.99**	8.76***	5.91***
Silk weight	5.01***	0.13 ^{ns}	2.66**	3.99***
Adult body weight	14.06***	1.57 ^{ns}	4.74***	4.03***
Forewing area	3.28**	- 1.82 ^{ns}	8.54***	5.20***
Adult longevity (without food)	15.76***	3.84***	7.93***	5.74***

^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

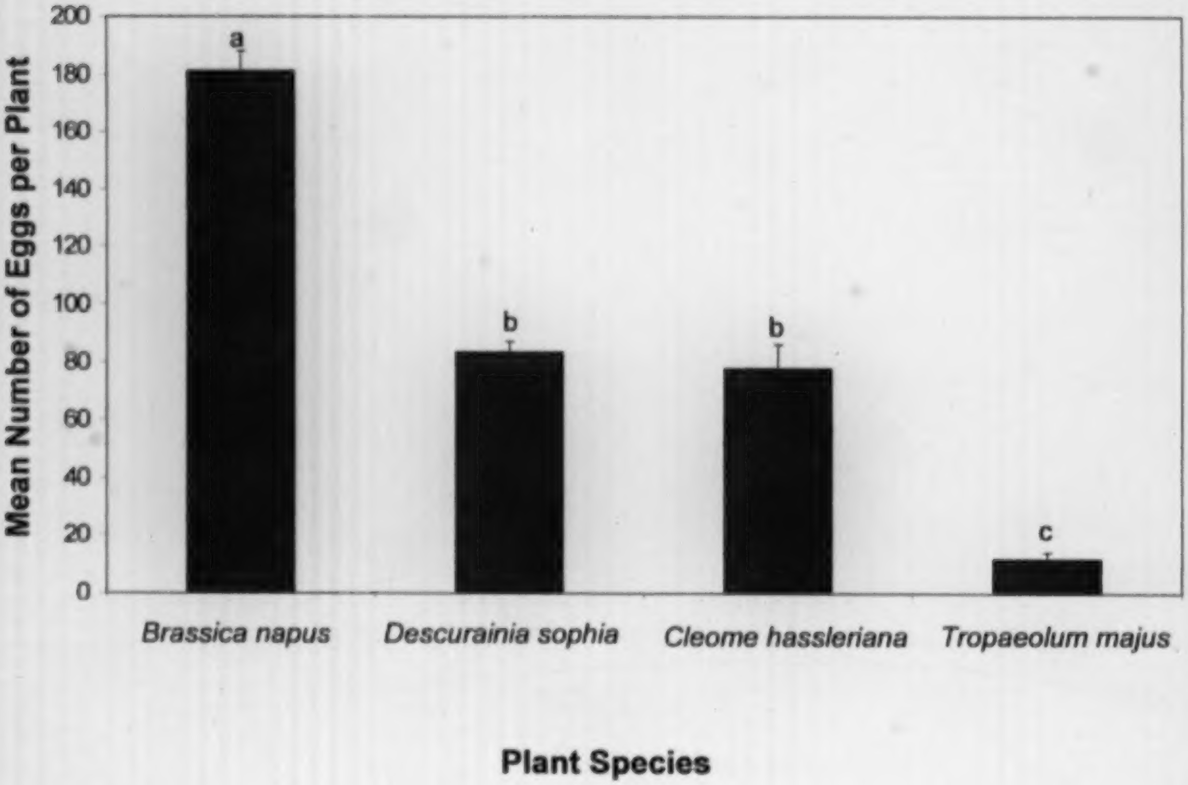

Figure 4-I. Oviposition preference (mean ± S.E.) by *Plutella xylostella* females in a choice situation (n = 10). Means sharing similar lowercase letters do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test.

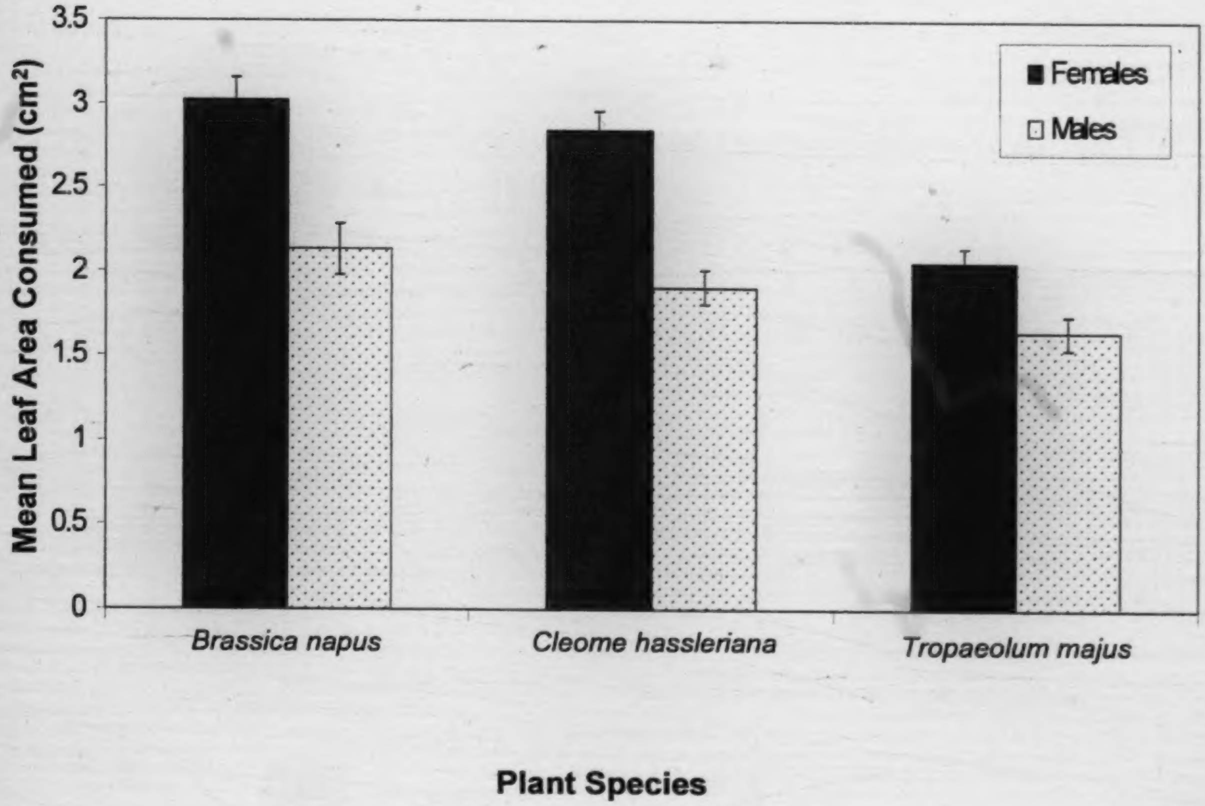

Figure 4-II. Mean leaf area (±S.E.) consumed per larva by a female (n =20) and male (n = 20) *Plutella xylostella* on *Brassica napus*, *Cleome hassleriana*, and *Tropaeolum majus*.

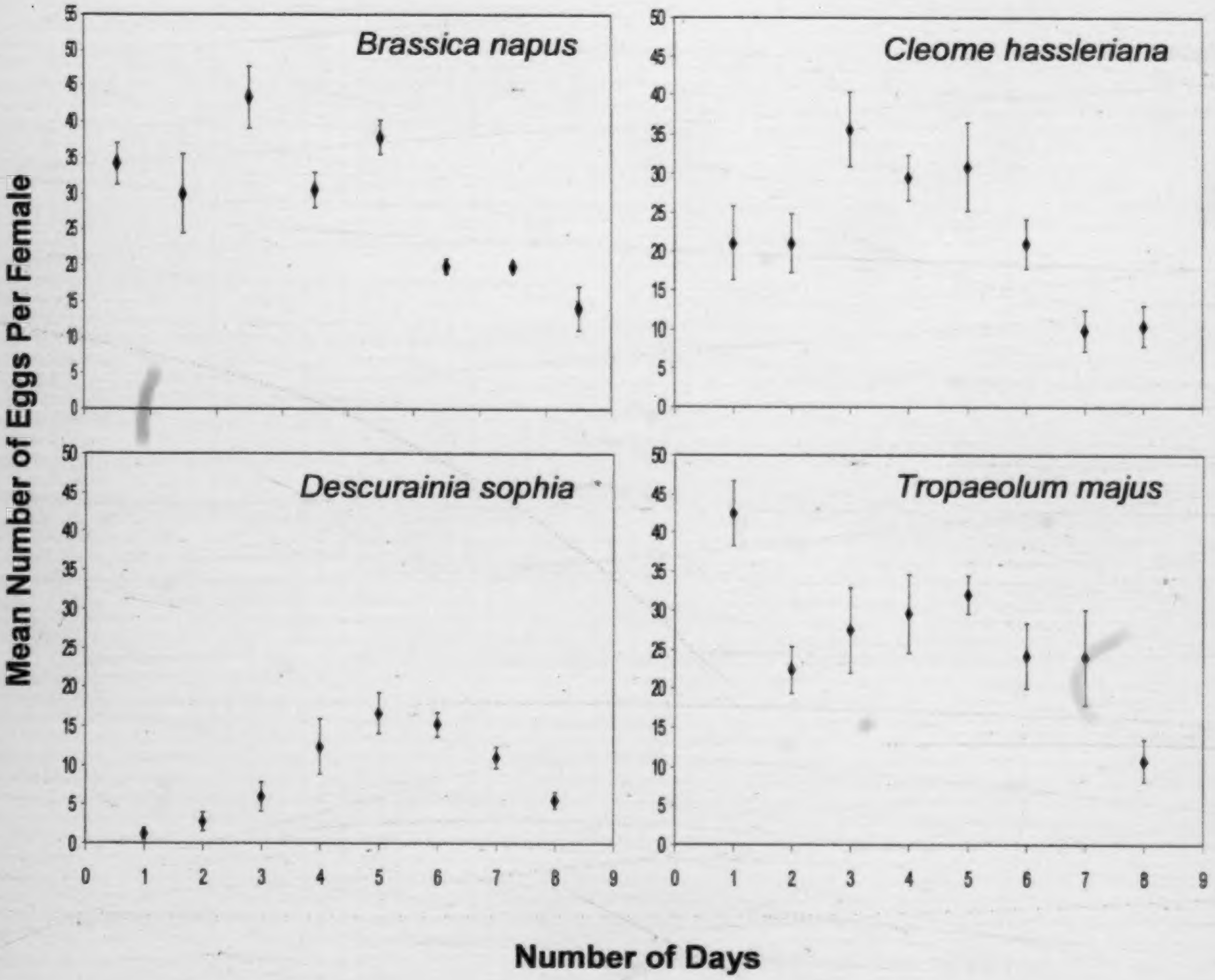

Figure 4-III. Mean oviposition (±S.E.) by new generation adults (n= 10) over an 8-day period when females were reared on *Brassica napus*, *Descurainia sophia*, *Cleome hassleriana* and *Tropaeolum majus.*

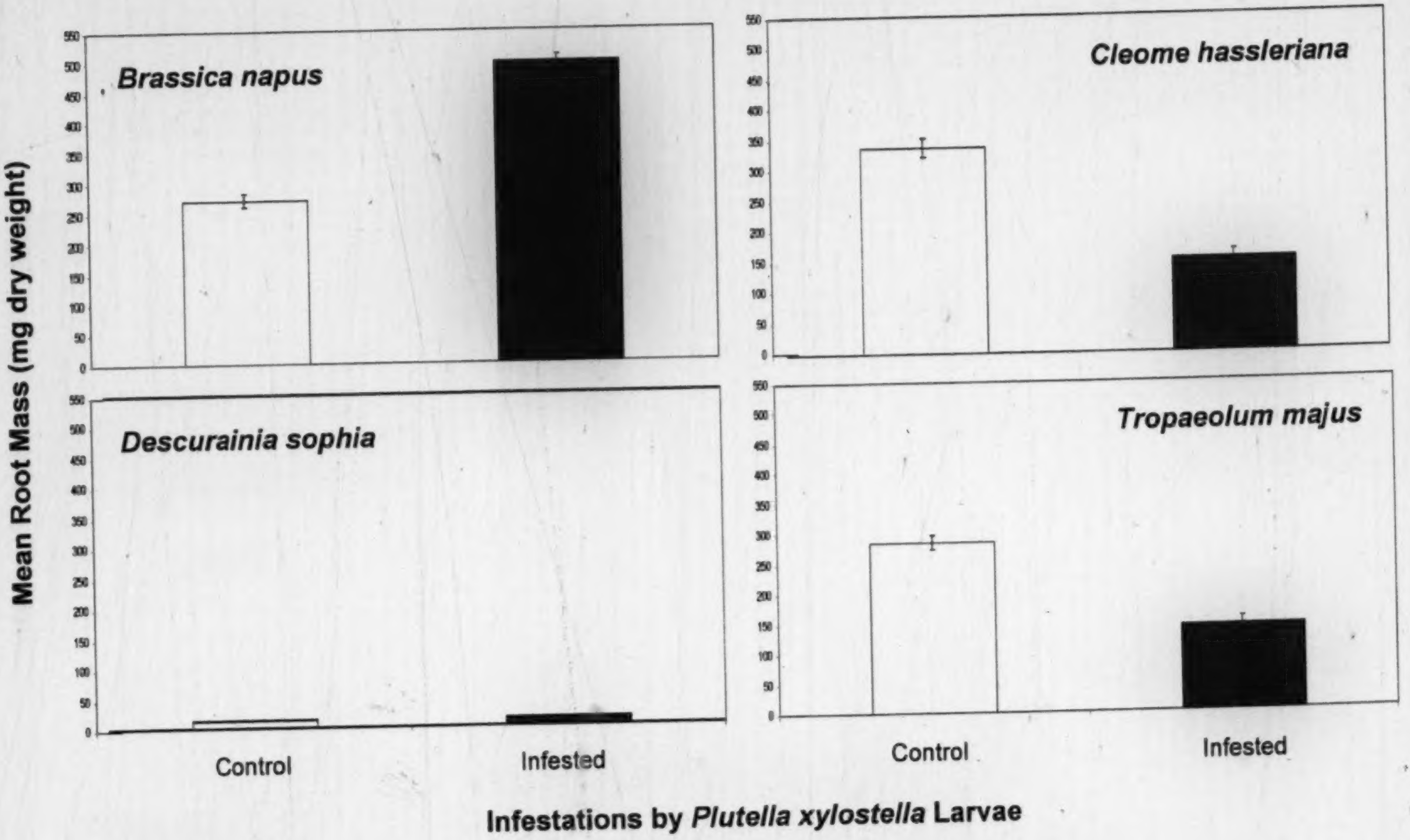

Figure 4-IV. Root mass development (mean ± S.E.) of Brassicaceae, Capparaceae and Tropaeolaceae species when infested (n =5) and non-infested (n = 5) by *Plutella xylostella*.

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Chapter 5:

Host Plant Nutritional Quality Affects the Performance of the Parasitoid *Diadegma insulare* as Mediated Through the Herbivore *Plutella xylostella*

5.1 Introduction

In terrestrial systems where herbivory is common, bottom-up effects of plants may be of crucial importance (Price et al., 1980; Polis and Strong, 1996; Ode, 2006). The interactions between herbivorous insects and their host plants are undoubtedly influenced by host plant quality that can be determined largely by variation in certain abiotic factors (Hunter and Price, 1992; Moon et al., 2000). Perhaps one of the most important factors that shapes these interactions is nutrient availability and the subsequent nutrient contents of host plant tissues. Hypotheses of 'plant stress' and 'plant vigor' have been proposed to predict the response of insect herbivores to soil nutrients, as mediated by host plant quality. Some studies have shown that insects perform better on vigorous plants than on their stressed counterparts (e.g., Fox et al., 1990; Price, 1991; Meyer and Root, 1996; Craig and Ohgushi, 2002; Dosdall et al., 2004), while others indicate that stressed plants frequently support higher densities of insect herbivores (e.g., White, 1969; Mattson, 1980; Jones and Coleman, 1988).

The effects of soil nutrient regimes are relatively well studied for herbivores, but studies focusing on the effects of plant fertilization on parasitoids are rare. A parasitoid has to deal with a variety of challenges for its success, including host habitat identification, host location, host acceptance, host suitability and host regulation.

Nutritional quality of host plants can be important at every step affecting parasitoid preference and performance indirectly or directly. Plants are known to indirectly influence the foraging efficiency of parasitoids, while for host acceptance, host suitability and host regulation plant impacts are mainly direct (Tumlinson et al., 1992; Poppy, 1997; Turlings and Benrey, 1998). A few experimental studies have indicated that abiotically induced changes in host plant quality can have strong effects on the performance of parasitoids. For example, *Anagrus armatus* (Ashmead) (Hymenoptera: Mymaridae) parasitized significantly more eggs of the salt marsh planthopper, *Pissonotus quadripustulatus* van Duzee (Homoptera: Delphacidae) on fertilized than on unfertilized plants (Moon et al., 2000). Plant nutritional quality can influence host size and most host-size models assume that larger hosts are superior to smaller hosts in terms of parasitoid fitness. The evidence in support of such host-size models comes mainly from studies of non-feeding hosts (e.g., eggs and pupae) that contain a fixed quantity of resources for idiobiont parasitoids (Salt, 1940; Arthur and Wylie, 1959; Charnov et al., 1981; King, 1989; Corrigan and Lashomb, 1990; Moon et al., 2000). In contrast to non-feeding hosts, insect herbivores continue to feed and develop after parasitization by koinobiont parasitoids until destructive consumption of their host (Jowyk and Smilowitz, 1978; Beckage and Riddiford, 1983; Sequeira and Mackauer, 1992; Harvey et al., 1995; Fox et al., 1996). Therefore, the quality of plant tissue consumed by herbivore hosts after parasitization may have stronger bottom-up influences on the performance of koinobionts than on idiobionts.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a specialist on Brassicaceae (Talekar and Shelton, 1993; Idris and Grafius, 1996; Sarfraz et al., 2006), a family of plants characterized by the presence of sulfur-containing secondary plant compounds, the glucosinolates (Mithen, 1992). *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) is a solitary koinobiont, host-specific larval endoparasitoid of *P. xylostella* and is one of its most important biological control agents in North America (Harcourt, 1960; Fitton and Walker, 1992; Sarfraz et al., 2005a). It parasitizes all four larval instars of *P. xylostella* and one host larva supports only one parasitoid larva. It kills and emerges from the pre-pupal stage of its host and spins its own cocoon inside the loosely woven cocoon of its host (Harcourt, 1960). Like other parasitoid species of herbivores, *D. insulare* utilizes plant cues to locate its *P. xylostella* hosts (Tumlinson et al., 1992; Turlings and Benrey, 1998, Ohara et al., 2003). The unique biochemistry of Brassicaceae, the oligophagous habit of *P. xylostella*, and host specialization of *D. insulare* provide an excellent model to investigate tritrophic interactions based on the nutritional quality of plants, but to my knowledge studies focusing on such interactions are rare.

In the present study, the effects of different soil fertility regimes were examined, viz. no added fertility, two levels of intermediate fertility and two levels of high fertility on *Brassica napus* L. (Brassicaceae), and on performance parameters of the parasitoid, *D. insulare*, developing within larvae of *P. xylostella* that were feeding upon leaf tissues of *B. napus*. Several fitness correlates directly related to parasitoid population dynamics (e.g., level of parasitism, survival, pre-imaginal developmental time, larval herbivory, pupal weight, adult body weight, longevity without food, forewing area, and hindwing area) were investigated on *P. xylostella* feeding on plants grown under five soil fertility regimes. A further objective of this study was to investigate how male and female *D.*

insulare respond to bottom-up forces when its *P. xylostella* host larvae were reared on host plants grown under different soil fertility treatments.

5.2 Materials and Methods

5.2.1 Insects and Plants

The laboratory colonies of *P. xylostella* and *D. insulare* were maintained on potted *B. napus* cv. Q2 plants at 22±0.5°C with 16h L: 8h D. Moths and wasps collected from commercial fields of *B. napus* in Alberta, Canada were added to the culture every summer to maintain genetic diversity.

Brassica napus plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium. Treatment plants were grown under four soil fertility regimes viz. 0.5, 1.0, 3.0, 5.0 g pot^{-1} of 20: 20: 20 (nitrogen: phosphorous: potassium) (Plant Products Co. Ltd., Brampton, Ontario, Canada) while control plants were grown without any added fertilizer. Fertilizer treatments were applied in two split applications to avoid any phytotoxic effects of higher concentrations: the first application was made after the second week of seed germination and the second application was made when plants were three weeks old. Each fertilizer treatment was applied after dissolving concentrate in 100 ml of tap water while control plants received 100 ml water only. Four-week-old plants were used for all experiments.

5.2.2 Intact Plant Study

5.2.2.1 *Diadegma insulare*: Parasitism and Survival

Parasitism and survival and development from egg to pupa were assessed in screened cages (40 x 40 x 80 cm), arranged on greenhouse benches in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 75 cages with 15 plants from each treatment. Fifty plants were infested with first-instar *P. xylostella* larvae at 10 larvae per plant by holding neonates carefully from their silk to avoid handling damage, while the remaining 25 plants served as uninfested controls for tissue nutrient analysis (see Chapter 2). Larvae were observed daily until they molted to second instars; neonates had no prior feeding experience. Five plants of each soil fertility treatment received two female and two male wasps ($\leq$ 3 days old) whereas the remaining five plants of each soil fertility served as controls infested with *P. xylostella* only (see Sarfraz et al., 2007); wasps were allowed to parasitize *P. xylostella* larvae for 24 h and were then removed from the cages. Plants were observed every 48 h and the numbers of surviving larvae were recorded, but daily observations were made when pupation began. Pupae were harvested, weighed within 24 h of pupation and kept individually in labeled transparent plastic cups until adult emergence.

It was assumed that mortality of *P. xylostella* larvae other than control mortality was caused by parasitoids and the percent parasitism was calculated using the following equation:

$$\text{Parasitism (\%)} = [(P_{di} \div L_t) \times 100] + M_c$$

where P_{di} = numbers of *D. insulare* pupae that developed, L_t = total numbers of *P. xylostella* larvae introduced to each cage, and M_c = percent corrected mortality determined by Schneider-Orelli's formula (Schneider-Orelli, 1947).

5.2.2.2 Tissue Nutrient Analysis

Leaves from each replicate uninfested control plant were collected at the end of the larval development experiment (15 to 17 days), air-dried at room temperature, ground and subjected to nutrient analysis. The AOAC-990.03 reference method was followed for determination of total nitrogen and sulfur (AOAC, 2003a) while calcium, phosphorous, potassium, magnesium and sodium were assessed by using AOAC-985.01 in Norwest Labs, Lethbridge, Canada (AOAC, 2003b).

5.2.3 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

This study was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D). Excised leaves taken from uninfested plants were placed on moist filter papers (9-cm diameter) in plastic containers covered with transparent ventilated lids. For each soil fertility treatment, 100 newly molted second-instar larvae ($\leq$ 24 h old) taken from the laboratory colony were parasitized (each larva was offered to female wasps individually and observed until parasitized) and introduced into individual plastic containers; a total of 500 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Developmental times from egg to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were also weighed using a Sartorius Supermicro® scale (Sartorius Inc., Edgewood, NY, USA). Adults were separated by sex and used in the longevity (without food), body weight, and forewing and hindwing area experiments.

To quantify levels of larval feeding, all leaves damaged by parasitized *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA). Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory.

Twenty females and 20 males of *D. insulare* reared from each treatment were used to determine their longevities without food. Specimens were placed in individual plastic containers in a growth chamber (22±0.5°C with 16h L: 8h D) and examined daily for survival or mortality. Wasps were weighed within 24 h of their death. Forewings and hindwings were carefully removed, glued onto paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA), and their areas were measured using Image J as described by Sarfraz et al. (2007).

5.2.4 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used as necessary to achieve normality and homoscedasticity before analysis (Steel et al., 1997), but untransformed means are presented graphically and in tables. Analyses of variance (ANOVA) (PROC GLM) for a completely randomized design were performed to assess the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). Pearson correlation coefficients (PROC CORR) were determined between pre-pupal development time and silk weight, pupal weight and silk weight, pupal weight and adult weight, pupal weight and longevity without food, herbivory and adult weight, adult weight and longevity, pupal weight and forewing area, adult weight and forewing area, and forewing area and hindwing area for both female and male specimens. T-tests (PROC TTEST) were performed for pair-wise comparisons between female and male insects for their developmental times, larval herbivory, pupal weights, silk weights, adult body weights, longevity (without food), forewing and hindwing areas when they were reared on plants grown with various fertilizer applications.

5.3 Results

5.3.1 *Diadegma insulare*: Parasitism and Survival

Brassica napus nutrient regimes significantly affected mean percent parasitism ($F_{4,36} = 3.67$, $P = 0.0133$). Parasitism occurrence was greatest when host larvae were reared on plants grown with 3.0 g fertilizer pot^{-1} (57.38 ± 5.84), and this significantly exceeded that for hosts reared on plants grown with 0 and 5.0 g fertilizer pot^{-1}, but not for hosts reared on plants grown with 0.5 and 1.0 g fertilizer pot^{-1} (Figure 5-I).

Host plant quality had a significant effect on avoidance/escape of *P. xylostella* from *D. insulare*; i.e., the numbers of individuals that successfully developed into *P. xylostella* pupae in the presence of wasps ($F_{4,36} = 5.41$, $P = 0.0016$). *Plutella xylostella* escape percentage was highest on plants fertilized at 1.0 g (32.00 ± 8.27), but it did not differ among plants grown with 0.0, 0.5, 3.0 and 5.0 g fertilizer (4.00 ± 2.67, 8.00 ± 3.27, 6.00 ± 1.63 and 4.00 ± 1.63 respectively).

Plant nutrient regime on which host *P. xylostella* were reared had significant effects on survival of *D. insulare* from egg to pupa ($F_{4,36}$ = 3.35, P = 0.0197), but non-significant effects on survival from pupa to adult ($F_{4,36}$ = 1.77, P = 0.1562) (Table 5-I). Survival from egg to pupa on plants grown with 3.0 g fertilizer pot^{-1} was significantly greater than on plants grown without any added fertilizer, but survival did not differ significantly among the other plant nutrient regimes (Table 5-I).

5.3.2 Tissue Nutrient Analysis

Soil fertility levels strongly influenced foliar nutrients (nitrogen: $F_{4,16}$ = 586.49, P < 0.0001; phosphorous: $F_{4,16}$ = 122.84, P < 0.0001; potassium: $F_{4,16}$ = 1081.75, P < 0.0001; sulfur: $F_{4,16}$ = 667.07, P < 0.0001; calcium: $F_{4,16}$ = 292.30, P < 0.0001; magnesium: $F_{4,16}$ = 88.58, P < 0.0001, and sodium: $F_{4,16}$ = 51.33, P < 0.0001) in *B. napus* (Chapter 2; Table 2-VIII).

5.3.3 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

Host plant nutrient quality on which *P. xylostella* larvae were reared significantly affected developmental times of *D. insulare* from egg to pre-pupa, pupa to adult, and egg to adult for both females ($F_{4,76}$ = 12.52, P < 0.0001; $F_{4,76}$ = 12.74, P < 0.0001 and $F_{4,76}$ = 9.98, P < 0.0001 respectively) and males ($F_{4,76}$ = 24.35, P < 0.0001; $F_{4,76}$ = 66.29, P < 0.0001 and $F_{4,76}$ = 22.81, P < 0.0001 respectively) (Table 5-II). Host plants grown with different soil fertility regimes significantly affected developmental times from pre-pupa to pupa for females ($F_{4,76} = 3.05$, $P = 0.0219$), but not for males ($F_{4,76} = 0.04$, $P = 0.9975$) (Table 5-II). Foliage consumption by *P. xylostella* larvae varied significantly when parasitized by female and male larvae of *D. insulare* ($F_{4,76} = 16.68$, $P < 0.0001$ and $F_{4,76} = 10.90$, $P < 0.0001$ respectively), and pupal weights differed significantly for females and males ($F_{4,76} = 4.25$, $P = 0.0037$ and $F_{4,76} = 3.07$, $P = 0.0213$ respectively) (Table 5-III). Silk weight of female *D. insulare* specimens was significantly affected by plant quality on which their hosts were reared for both females ($F_{4,76} = 9.98$, $P < 0.0001$) and males ($F_{4,76} = 5.97$, $P = 0.0003$) (Table 5-III). Host plant quality significantly affected adult body weight and longevity (without food) of females ($F_{4,76} = 23.26$, $P < 0.0001$ and $F_{4,76} = 37.73$, $P < 0.0001$ respectively) and males ($F_{4,76} = 2.26$, $P = 0.0707$ and $F_{4,76} = 59.66$, $P < 0.0001$ respectively) (Table 5-IV). Forewing areas varied significantly for females ($F_{4,76} = 7.48$, $P < 0.0001$) and males ($F_{4,76} = 2.71$, $P = 0.0360$). Plant quality strongly affected hindwing areas of males but not of females ($F_{4,76} = 6.04$, $P = 0.0003$ and $F_{4,76} = 1.55$, $P = 0.1972$ respectively) (Table 5-IV).

Female and male development of *D. insulare* from egg to pre-pupa was fastest when hosts were reared on plants grown with 0.5 and 3.0 g fertilizer pot^{-1}. Both female and male pupae developed slowest on plants grown under 0.5 g soil fertility. Egg to adult development was faster on plants grown with 5.0 g fertilizer than on plants grown with 0.0, 0.5 and 1.0 g fertilizer (Table 5-II). *Plutella xylostella* larvae harboring female *D. insulare* consumed the largest leaf area when no fertilizer additions were made, whereas least foliage consumption occurred on plants grown with 5.0 g fertilizer pot^{-1} (Table 5-III). Female pupae were heavier when their *P. xylostella* hosts were reared on plants grown with 1.0 g fertilizer than on plants grown with 0.0 and 5.0 g fertilizer, but weights of male pupae were significantly lighter when host larvae were reared on plants fertilized at 1.0 than at 3.0 g pot^{-1} (Table 5-III). Female specimens reared on *P. xylostella* hosts that consumed highly fertilized plants (3.0 and 5.0 g pot^{-1}) produced significantly more silk than those from hosts that consumed plants grown with less fertilizer (0.0 and 0.5 g pot^{-1}). Males produced more silk when parasitized *P. xylostella* larvae were fed on plants grown with 0.5 g fertilizer than on plants grown without any added fertilizer (Table 5-III).

Heaviest females of *D. insulare* were produced on *P. xylostella* hosts reared on plants fertilized at 3.0 g pot^{-1}; however, body weights of males did not differ significantly when reared on plants grown under various soil fertility regimes (Table 5-IV). Female wasps reared on *P. xylostella* hosts that consumed unfertilized plants lived for the shortest time in the absence of food whereas males reared on plants grown at 3.0 g fertilizer lived the longest time. Female and male wasps reared on larvae that fed on plants grown with 3.0 and 5.0 g fertilizer had the largest forewings, respectively. Regardless of fertilizer treatments, hindwing areas for females did not differ significantly; males with largest hindwings were from larvae reared on plants grown at 5.0 g fertilizer pot^{-1} (Table 5-IV).

For female specimens reared on *P. xylostella* hosts that consumed host plants varying in quality, significant correlations were found between pre-pupal development time and silk weight ($r = 0.31$, $P = 0.0020$), pupal weight and silk weight ($r = 0.39$, $P < 0.0001$), pupal weight and longevity ($r = 0.21$, $P = 0.0407$), pupal weight and forewing area ($r = 0.41$, $P < 0.0001$), adult weight and longevity ($r = 0.23$, $P = 0.0201$), adult weight and forewing area ($r = 0.25$; $P = 0.0104$), and forewing area and hindwing area ($r = 0.25$, $P = 0.0122$), but non-significant correlations existed between herbivory and adult weight ($r = -0.07$, $P = 0.5176$), and pupal weight and adult weight ($r = 0.16$, $P = 0.1035$).

For males, significant correlations were found between pupal weight and adult weight ($r = 0.29$, $P = 0.0034$), pupal weight and forewing area ($r = 0.37$, $P = 0.0001$), forewing area and hindwing area ($r = 0.42$, $P < 0.0001$), but non-significant correlations existed between pre-pupal development time and silk weight ($r = 0.12$, $P = 0.2410$), pupal weight and silk weight ($r = -0.01$, $P = 0.9429$), pupal weight and longevity ($r = 0.12$, $P = 0.2341$), adult weight and longevity ($r = 0.05$, $P = 0.6364$), adult weight and forewing area ($r = 0.10$, $P = 0.3160$), and herbivory and adult weight ($r = 0.11$, $P = 0.2843$).

Female and male *D. insulare* exhibited significant differences in only a few life history traits when reared on plants receiving the same treatment (Table 5-V). Pupa to adult development was slower for females than their male counterparts when parasitized *P. xylostella* larvae were reared on plants treated at 1.0, 3.0 and 5.0 g fertilizer pot^{-1}. Female pupae were heavier than male pupae when parasitized host larvae were reared on plants grown with 1.0 g fertilizer whereas male pupae were heavier than female pupae when reared on plants fertilized at 5.0 g. Females were heavier with larger forewings and hindwings than their male counterparts when parasitized *P. xylostella* larvae were reared on plants grown with 3.0 g fertilizer pot^{-1}. Males reared on plants grown with 0.0 and 3.0 g fertilizer lived longer without food than females, but females from 0.5 g soil fertility plants were more long lived than their male counterparts (Table 5-V).

5.4 Discussion

This is the first study of its kind to investigate the effects of a range of nutrient regimes on several fitness correlates of the koinobiont *D. insulare*. Variation in soil fertility influenced the nutritional quality of host plants and this in turn affected the performance of *D. insulare*. In general, *D. insulare* performed best on *P. xylostella* larvae that developed on plants grown with 3.0 g fertilizer; these plants had 2.06-, 3.77-, and 1.02- fold more nitrogen, phosphorous and potassium respectively than plants grown without any added fertilizer. By contrast, my earlier research indicated that overall performance of non-parasitized *P. xylostella* hosts was better on plants grown at 1.0 g fertilizer than on other treatment plants (Chapter 2; Sarfraz et al., 2005b). Parasitism and survival of *D. insulare* varied considerably among the tested soil fertility regimes on which host *P. xylostella* larvae were reared with most on plants grown with 3.0 g fertilizer and least on unfertilized plants. Some previous evidence exists that parasitism rates by *D. insulare* were significantly higher on high-nitrogen collard plants (*Brassica oleracea* L. var. *acephala*) than on low-nitrogen plants (Fox et al. 1990). Contrary to the present findings, Fox et al. (1996) found that *D. insulare* survival/adult emergence was higher on "low-fertilizer" collard plants than on "high-fertilizer" plants. However, I was unable to determine how much fertilizer was actually used to grow "low-fertilizer" and "high-fertilizer" plants in their study, and the authors did not provide data on tissue nutrient contents of their treatment plants.

Plutella xylostella escape from *D. insulare* was highest on plants grown at 1.0 g fertilizer. This could be attributed to both physical and physiological defensive mechanisms mediated by host plant quality. Herbivore hosts can sometimes physically deter parasitoids successfully, for instance by wriggling, walking away, jumping, kicking, dropping off the plant or secreting defensive chemicals (Godfray, 1994). *Plutella xylostella* larvae can deter parasitism primarily by wriggling and dropping off the plant, where they can hang suspended on silken threads until the parasitoid departs (Sarfraz, personal observation). Similarly, *Myzus persicae* (Sulzer) (Homoptera: Aphididae) successfully escaped from *Aphelinus flavus* Nees (Hymenoptera: Aphelinidae) in 58% of oviposition attacks by walking away and dropping off the plant (Wilbert, 1967; Jansson, 2003). The immune systems of herbivore hosts can also defend against parasitism (e.g. through encapsulation), and kill the parasitoid eggs or larvae. Plant quality can affect the ability of a herbivore to encapsulate parasitoid eggs (Cheng, 1970; Benrey and Denno, 1997); the success of encapsulation depends on the vigor of the herbivore, and this can be influenced by host plant nutritional quality and suitability (Muldrew, 1953; van den Bosch, 1964; Vinson and Barbosa, 1987). A plant with toxins or low nutritional quality that renders the herbivore host weakened may suppress its immune system and thereby benefit the parasitoid. In the present study, physiological mechanisms were also likely involved because relatively high numbers (ca. 8 to 10%) of *P. xylostella* larvae that were individually parasitized for life history trait experiments (section 5.2.3) developed into *P. xylostella* pupae instead of *D. insulare* when reared on plants grown at 1.0 g fertilizer. By contrast, larvae of *P. xylostella* reared on plants subjected to other soil fertility treatments escaped parasitism by *D. insulare* less frequently (0 to 4%).

Sarfraz et al. (2005b) determined that among these soil fertility treatments, the application rate of 1.0 g pot^{-1} was maximal for *P. xylostella*. Mean numbers of *P. xylostella* eggs per plant and percent survival from neonate to pupa were greatest on plants fertilized at 1.0 g pot^{-1}. In addition, larval and pupal development rates were fastest when plants were fertilized at this rate. Here I report that this rate is also maximal for enabling *P. xylostella* to escape parasitism by *D. insulare*. Evidently the ability of *P. xylostella* females to select host plants with maximal nutrient levels confers benefits that extend beyond merely rapid developmental rates of their offspring; maximal plant nutrients enhance survival of their offspring whether or not their larvae are under attack by *D. insulare*.

Plant quality on which *P. xylostella* hosts were reared significantly affected developmental times of both female and male *D. insulare*. Nutritional sufficiency and quality of the plant can affect parasitoid development (Vinson and Iwantsch, 1980). For instance, performance of the parasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) varied when parasitized *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) hosts were reared on artificial diets of different quality; parasitoids took longer to develop and experienced higher mortality when reared in hosts that were fed on low-quality diet (Harvey et al., 1995). For female specimens of *D. insulare*, slowest development from egg to pre-pupa and from pupa to adult occurred on host larvae reared on plants grown with 0.0 and 0.5 g fertilizer respectively. According to the "Slow Growth-High Mortality Hypothesis", individuals feeding on larvae reared on these treatment plants may have an extended window of vulnerability to natural enemies (Benrey and Denno, 1997). On the other hand, *D. insulare* egg to adult development was shorter (about one day) when parasitized *P. xylostella* hosts were reared on plants grown with high soil fertility than on plants grown with no or intermediate soil fertility. Development of *P. xylostella* from egg to adult is more rapid in tropical than in temperate regions, with as many as 20 generations per year possible in the tropics (Vickers et al., 2004; Sarfraz et al., 2005a). The one-day difference in development time of *D. insulare* at higher levels of soil fertility may result in an additional generation per year in tropics, and thereby enhance the biological control of *P. xylostella* in those regions.

Increased nitrogen and other nutrients can lead to unbalanced amino acid profiles and large concentrations of organic acids in plant tissues; consequently, such diets can be detrimental and even toxic to insects (Brodbeck et al., 1990). Larvae consumed more foliage of plants grown at low and intermediate soil fertility levels than at high fertility. Substantial evidence exists that insects need to compensate for lowered concentrations of nitrogen (and possibly other nutrients) by increasing food intake (Slansky and Feeny, 1977; Berner et al., 2005). The plants grown at different soil fertility regimes influenced the pupal weights, silk weights, adult body weights, longevities, and sizes of forewings and hindwings of *D. insulare* when parasitized *P. xylostella* hosts were reared on plants subjected to various soil fertility treatments. Growth is governed by the rate of biomass gain, the efficiency of conversion of food into tissue, and the metabolic costs of maintenance (Calow, 1977; Sequeira and Mackauer, 1992). For a given set of environmental conditions, efficient growth should yield maximum biomass at minimum metabolic cost in the shortest developmental time. Parasitoid larval growth provides a direct measure of host quality because it reflects the nutritional interactions between the two species throughout the course of parasitism. Because nutrient reserves accumulated by larvae feeding on different quality plants may not be the same, these differences can have consequences on adult life-history parameters, including fecundity, longevity (Boggs, 1981; Sequeira and Mackauer, 1992) and body size. Body size is an important fitness index because it often affects reproductive success through variations in fecundity, longevity, dispersal, searching efficiency, and host handling strategies (Charnov and Skinner, 1984; Visser, 1994; Kazmer and Luck, 1995).

Table 5-I. Mean percent survival (± S.E.) of *Diadegma insulare* from egg to pupa and from pupa to adult when parasitized host *Plutella xylostella* larvae (n = 10) were reared on intact *Brassica napus* plants grown under various fertility regimes.

Fertilizer application rate (g pot^{-1})	Survival (%)	
	Egg to Pupa	Pupa to Adult
0.0	32.00^{b}	64.00^{a}
	(4.90)	(8.27)
0.5	42.00^{ab}	68.48^{a}
	(4.42)	(7.37)
1.0	42.00^{ab}	77.26^{a}
	(1.33)	(6.27)
3.0	58.00^{a}	72.86^{a}
	(6.46)	(5.87)
5.0	56.00^{ab}	52.50^{a}
	(5.81)	(8.13)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 5-II. Mean (± S.E.) developmental times of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized host *Plutella xylostella* larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Egg to pre-pupa (days)	Female	7.90 ^{a}	6.40 ^{c}	7.30 ^{ab}	6.40 ^{c}	6.70 ^{bc}
		(0.22)	(0.19)	(0.11)	(0.21)	(0.18)
	Male	8.30 ^{a}	6.05 ^{c}	7.20 ^{b}	6.40 ^{c}	6.60 ^{bc}
		(0.27)	(0.11)	(0.09)	(0.11)	(0.18)
Pre-pupa to pupa (days)	Female	1.60 ^{ab}	1.20 ^{b}	1.70 ^{ab}	1.90 ^{a}	1.60 ^{ab}
		(0.18)	(0.09)	(0.11)	(0.19)	(0.11)
	Male	1.35 ^{a}	1.40 ^{a}	1.40 ^{a}	1.40 ^{a}	1.40 ^{a}
		(0.11)	(0.12)	(0.11)	(0.17)	(0.18)
Pupa to adult (days)	Female	7.20 ^{b}	8.65 ^{a}	7.70 ^{b}	7.30 ^{b}	7.00 ^{b}
		(0.27)	(0.21)	(0.11)	(0.11)	(0.10)
	Male	7.00 ^{bc}	9.15 ^{a}	7.20 ^{b}	6.70 ^{bc}	6.60 ^{c}
		(0.15)	(0.17)	(0.09)	(0.18)	(0.11)
Egg to adult (days)	Female	16.70 ^{a}	16.25 ^{ab}	16.70 ^{a}	15.60 ^{bc}	15.30 ^{c}
		(0.27)	(0.16)	(0.11)	(0.21)	(0.18)
	Male	16.65 ^{a}	16.60 ^{a}	15.80 ^{a}	14.50 ^{b}	14.60 ^{b}
		(0.26)	(0.29)	(0.17)	(0.24)	(0.11)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 5-III. Mean (± S.E.) foliage consumption, pupal weights and silk weights of *Diadegma insulare* female (n = 20) and male (n =20) specimens when parasitized *Plutella xylostella* host larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Foliage consumed (cm^2)	Female	2.96 ^{a}	2.45 ^{a}	2.37 ^{ab}	1.83 ^{bc}	1.35 ^{c}
		(0.23)	(0.19)	(0.14)	(0.09)	(0.04)
	Male	2.64 ^{a}	2.50 ^{ab}	1.87 ^{bc}	1.56 ^{c}	1.17 ^{c}
		(0.09)	(0.35)	(0.13)	(0.12)	(0.04)
Pupal weight (mg)	Female	3.91 ^{b}	4.07 ^{ab}	4.43 ^{a}	4.37 ^{ab}	3.94 ^{b}
		(0.19)	(0.12)	(0.06)	(0.06)	(0.05)
	Male	4.12 ^{ab}	3.89 ^{ab}	3.76 ^{b}	4.19 ^{a}	4.15 ^{ab}
		(0.09)	(0.14)	(0.12)	(0.08)	(0.07)
Silk weight (mg)	Female	0.48 ^{b}	0.54 ^{b}	0.56 ^{ab}	0.64 ^{a}	0.63 ^{a}
		(0.03)	(0.03)	(0.01)	(0.01)	(0.02)
	Male	0.57 ^{c}	0.71 ^{a}	0.60 ^{bc}	0.65 ^{abc}	0.68 ^{ab}
		(0.02)	(0.04)	(0.02)	(0.02)	(0.02)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 5-IV. Mean (± S.E.) adult body weights, longevity without food, forewing and hindwing areas of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized *Plutella xylostella* host larvae were reared on leaf tissue of *Brassica napus* plants grown under five different soil fertility regimes.

		Fertilizer application rate (g pot^{-1})				
Biological parameters		0.0	0.5	1.0	3.0	5.0
Adult body weight (mg)	Female	0.72 ^{c}	1.10 ^{ab}	0.96 ^{b}	1.26 ^{a}	0.73 ^{c}
		(0.04)	(0.03)	(0.01)	(0.09)	(0.03)
	Male	0.84 ^{a}	0.94 ^{a}	0.98 ^{a}	1.02 ^{a}	0.99 ^{a}
		(0.06)	(0.03)	(0.04)	(0.04)	(0.05)
Longevity without food (days)	Female	2.00 ^{b}	3.50 ^{a}	3.70 ^{a}	3.70 ^{a}	3.70 ^{a}
		(0.21)	(0.11)	(0.11)	(0.11)	(0.11)
	Male	2.95 ^{c}	1.65 ^{d}	3.60 ^{b}	4.40 ^{a}	3.60 ^{b}
		(0.09)	(0.15)	(0.11)	(0.18)	(0.11)
Forewing area (cm^{2})	Female	0.048 ^{b}	0.048 ^{b}	0.053 ^{ab}	0.057 ^{a}	0.051 ^{b}
		(0.001)	(0.001)	(0.002)	(0.001)	(0.001)
	Male	0.049 ^{ab}	0.044 ^{ab}	0.043 ^{b}	0.047 ^{ab}	0.052 ^{a}
		(0.002)	(0.001)	(0.002)	(0.003)	(0.002)
Hindwing area (cm^{2})	Female	0.029 ^{a}	0.020 ^{a}	0.023 ^{a}	0.029 ^{a}	0.026 ^{a}
		(0.006)	(0.00)	(0.001)	(0.001)	(0.001)
	Male	0.021 ^{ab}	0.017 ^{b}	0.022 ^{ab}	0.022 ^{ab}	0.026 ^{a}
		(0.002)	(0.001)	(0.001)	(0.001)	(0.001)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 5-V. Pair-wise comparisons (t-values) between female (n = 20) and male (n = 20) *Diadegma insulare* for some key life history parameters when parasitized host *Plutella xylostella* larvae were reared on *Brassica napus* grown under similar fertility treatment.

Biological parameters	Fertilizer application rate (g pot^{-1})				
	0.0	0.5	1.0	3.0	5.0
Development time (egg to pre-pupa)	-1.15^{ns}	1.54^{ns}	0.72^{ns}	0.00^{ns}	0.39^{ns}
Development time (pre-pupa to pupa)	1.17^{ns}	-1.34^{ns}	1.95^{ns}	1.97^{ns}	0.93^{ns}
Development time (pupa to adult)	0.66^{ns}	-1.87^{ns}	3.58***	2.89**	2.63*
Development time (egg to adult)	0.13^{ns}	-1.05^{ns}	4.47***	3.49**	3.31**
Herbivory (per larva)	1.31^{ns}	-0.12^{ns}	2.64**	1.82^{ns}	3.27**
Pupal weight	-0.98^{ns}	0.97^{ns}	5.02***	1.74^{ns}	-2.61*
Silk weight	-2.48*	-3.79***	-2.43*	-0.42^{ns}	-1.81^{ns}
Adult body weight	-1.62^{ns}	3.94***	-0.44^{ns}	2.47*	-4.73***
Adult longevity (without food)	-4.25***	9.90***	0.65^{ns}	-3.31**	0.65^{ns}
Forewing area	-0.60^{ns}	1.54^{ns}	3.68***	3.71***	-0.12^{ns}
Hindwing area	1.19^{ns}	3.86***	0.50^{ns}	4.48***	0.22^{ns}

^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

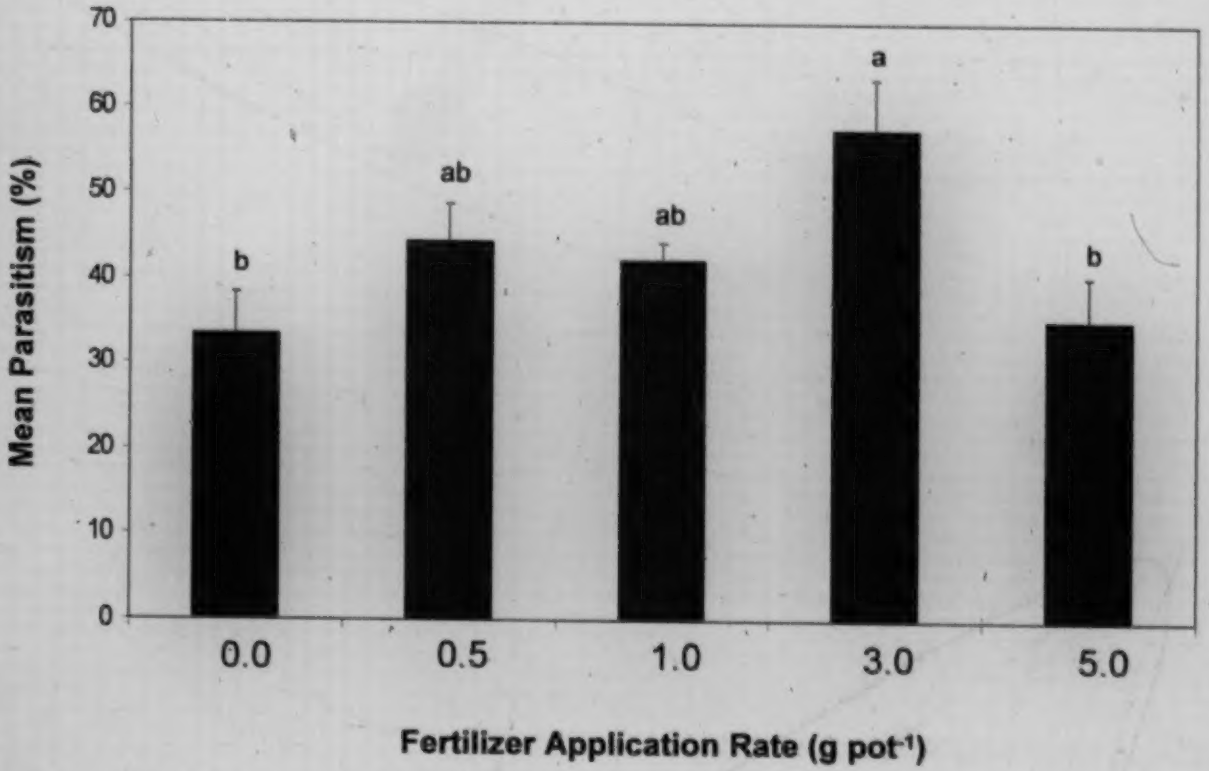

Figure 5-I. Parasitism of *Plutella xylostella* larvae by *Diadegma insulare* on intact plants in no-choice tests. Means and standard errors are presented; means with different lowercase letters are significantly different from each other (ANOVA and Tukey's mean separation, $P = 0.05$).

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Chapter 6:

Host Plant Genotype of the Herbivore *Plutella xylostella* Affects the Performance of its Parasitoid *Diadegma insulare* [‡]

6.1 Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of brassicaceous crops worldwide (Talekar and Shelton, 1993; Sarfraz et al., 2006). It is a "leader" among difficult to control field crop insect pests owing to its rapid development of high levels of resistance to various insecticides (Mota-Sanchez et al., 2002; Sarfraz and Keddie, 2005). Consequently, increased efforts worldwide have been undertaken to develop integrated management strategies for its control, based principally on manipulation of its parasitoids. Although over 135 parasitoid species are known to attack various life stages of *P. xylostella*, most control worldwide is achieved by relatively few species belonging to the hymenopteran ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus* (see Sarfraz et al., 2005).

Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) is a solitary koinobiont, host-specific larval endoparasitoid of *P. xylostella* and is one of its most important biological control agents with a range extending from the Nearctic to northern Neotropical regions (Harcourt, 1960; Fitton and Walker, 1992; Idris and Grafius, 1996 2001; Braun et al., 2004; Sarfraz et al., 2005). In 1992, *D. insulare* accounted for ca. 30 and 45% of the total parasitism in canola fields in Alberta and Saskatchewan respectively (Braun et al., 2004). In New York, *D. insulare* resulted in 46.5% of the total *P. xylostella* parasitism from 1979 to 1994 (Shelton et al., 2002). In Florida, its contribution was 55 to 90% to the total *P. xylostella* parasitism recorded in cabbage crops during 1996 and 1997 (Hu et al., 1998). From 1994 to 2003, *P. xylostella* parasitism by *D. insulare* was 62 to 82% in Minnesota cabbage fields (Wold-Burkness et al., 2005).

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Compared with other parasitoids, *D. insulare* is an efficient host searcher (Xu et al., 2001; Wang and Keller, 2002). It parasitizes all four larval instars of *P. xylostella*; it kills and emerges from the pre-pupal stage of its host and spins its own cocoon inside the loosely woven cocoon of its host (Harcourt, 1960; Putnam 1968). The number of generations per year of *D. insulare* corresponds to the number of generations of *P. xylostella* as one host larva supports only one parasitoid larva (Putnam, 1968; Sourakov and Mitchell, 2000). However, despite its importance as a biological control agent, extensive research on factors affecting its life history traits is uncommon. Few previous studies have investigated the impacts of the host plant genotype utilized by its *P. xylostella* hosts on the fitness and developmental biology of *D. insulare*. For instance, Idris and Grafius (1996) reared *P. xylostella* on excised leaves of various cultivated and wild Brassicaceae, exposed them to *D. insulare* and measured parasitism rates, development times, and sex ratios.

My research was designed to build upon previous studies and provide detailed insights on some key life history traits of both male and female *D. insulare* when its *P. xylostella* host larvae were reared on various host plant species and cultivars. This study focused on determining effects on *D. insulare* of eight Brassicaceae commonly grown in various crucifer-growing areas worldwide, and which serve as hosts for *P. xylostella*. It is usually assumed that proteins conferring herbicide tolerance should not affect insect herbivores or their natural enemies, but recent research by Sarfraz et al. (2007) demonstrated that *P. xylostella* performance varied considerably on conventional and herbicide-tolerant canola (*Brassica napus* L.) along with other tested Brassicaceae. Therefore the same plant genotypes were used to investigate their potential bottom-up effects, if any, on the third trophic level. Several key life history parameters directly related to parasitoid population dynamics (e.g., parasitism, survival, pre-imaginal developmental time, larval herbivory, pupal weight, adult body weight, longevity without food, forewing area, and hindwing area) were investigated on all eight Brassicaceae. A further objective of this study was to provide detailed comparison between parasitized and unparasitized *P. xylostella* for defoliation when specimens were reared on tested plant genotypes.

6.2 Materials and Methods

6.2.1 Insects and Plants

The laboratory colonies of *P. xylostella* and *D. insulare* were maintained on potted *B. napus* cv. Q2 plants at 22±0.5°C with 16h L: 8h D. Moths and wasps collected from different fields in Alberta, Canada were added to the culture every summer to maintain genetic diversity.

Eight Brassicaceae, namely *B. napus* cv. Q2 (susceptible to both glufosinate ammonium and glyphosate herbicides), *B. napus* cv. Liberty (resistant to glufosinate ammonium), *B. napus* cv. Conquest (resistant to glyphosate), *B. rapa* L. cv. Reward, *B. juncea* (L.) Czern. cv. Cutlass, *B. carinata* L. (Accession No. BCA-003), *B. oleracea* L. cv. Red Acre, and *Sinapis alba* L. (Accession No. SAL-004) were grown under greenhouse conditions. Plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium and fertilized with 20: 20: 20 (N: P: K) at 0.5 g per pot when plants were two to three weeks old. Four-week-old plants were used for all experiments.

6.2.2 Intact Plant Study: Survival and Parasitism

Survival of *D. insulare* from egg to pupa was assessed in screened cages (40 x 40 x 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 80 cages with 10 plants from each species/cultivar. All plants were infested with first-instar *P. xylostella* larvae at 10 larvae per plant by holding neonates carefully from their silk to avoid handling damage; neonates had no prior feeding experience. Larvae were observed daily until they molted to second instars. Five plants of each genotype received two female and two male wasps (≤ 3 days old) whereas the remaining five plants served as controls (see Chapter 3); wasps were allowed to parasitize *P. xylostella* larvae for 24 h and were then removed from the cages. Plants were observed every 48 h and the numbers of surviving individuals were recorded, but daily observations were made when pupation began. *Diadegma insulare* pupae were harvested, weighed within 24 h of pupation and kept individually in labeled transparent plastic cups until adult emergence.

It was assumed that mortality of *P. xylostella* larvae other than control mortality was caused by parasitoids and the percent parasitism was calculated using the following equation:

$$\text{Parasitism (\%)} = [(P_{di} \div L_t) \times 100] + M_c$$

where P_{di} = numbers of *D. insulare* pupae that developed, L_t = total numbers of *P. xylostella* larvae introduced to each cage, and M_c = percent corrected mortality determined by Schneider-Orelli's formula (Schneider-Orelli, 1947).

6.2.3 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

This study was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D). Excised leaves were placed on moist filter papers (9-cm diameter) in plastic containers covered with transparent ventilated lids. For each plant genotype, 100 second-instar larvae (≤ 1 day old) taken from the laboratory colony were parasitized (each larva was offered to female wasps individually and observed until parasitized) and introduced into individual plastic containers; a total of 800 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Parasitoid developmental times from egg to pre-pupa and from pre-pupa to pupa were recorded. Wasp pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were also weighed using a Sartorius Supermicro® scale (Sartorius Inc., Edgewood, NY, USA). Adult *D. insulare* were sexed and used in the longevity (without food), body weight, and forewing and hindwing area experiments.

To quantify levels of larval feeding, all leaves damaged by parasitized and unparasitized (control) *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA). Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory as described by Sarfraz et al. (2007). Feeding comparison started with the second instar and continued until larvae stopped feeding just prior to the pre-pupal stage.

Twenty females and 20 males of *D. insulare* reared from each plant taxon were used to determine their longevities without food. Specimens were placed in individual plastic containers and examined daily for survival or mortality. Wasps were weighed within 24 h of their death. Forewings and hindwings were carefully removed, glued onto a paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA), and their areas were measured using Image J as described by Sarfraz et al. (2007).

6.2.4 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used as necessary to achieve normality and homoscedasticity in data before analysis (Steel et al., 1997), but untransformed means are presented graphically and in tables. Analyses of variance (ANOVA) (PROC GLM) for a completely randomized design were performed to test the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). Correlations (PROC CORR) were determined between larval herbivory and pupal weight, herbivory and silk weight, herbivory and adult weight, pupal weight and silk weight, pupal weight and adult weight, pupal weight and longevity without food, adult weight and longevity, pupal weight and forewing area, pupal weight and hindwing area, and forewing area and hindwing area for both female and male specimens. T-tests (PROC TTEST) were performed for pair-wise comparisons between female and male *D. insulare* for their developmental times, larval herbivory, pupal weights, silk weights, adult body weights, longevity (without adult food), forewing and hindwing areas when they were reared on various Brassicaceae. Data on foliage consumption by female and male larvae were averaged, and PROC TTEST was performed for pair-wise comparisons between parasitized and non-parasitized *P. xylostella* larvae on each plant genotype.

6.3 Results

6.3.1 Intact Plant Study: Survival and Parasitism

Species and cultivars on which parasitized *P. xylostella* larvae were reared had significant effects on survival of *D. insulare* from egg to pupa (F = 4.12; df = 7; P = 0.0009), and from pupa to adult (F = 2.85; df = 7; P = 0.0119) (Table 6-I). Survival of *D. insulare* from egg to pupa on *S. alba* was significantly greater than on *B. napus* cvs. Q2 and Liberty, but survival did not differ significantly among the other plant genotypes tested (Table 6-I). Mean percent survival from pupa to adult was greatest on *B. oleracea* and least on *B. carinata* (88.45 ± 2.71 (S.E.) and 55.26 ± 3.66, respectively). Pupa to adult survival of *D. insulare* was statistically similar when parasitized host larvae were reared on *B. napus* cvs. Q2, Liberty and Conquest, *B. juncea*, *B. rapa* and *S. alba* (Table 6-I).

Brassicaceae genotype significantly affected mean percent parasitism ($F = 11.69$; df = 7; $P < 0.0001$) (Figure 6-I). Parasitism occurrence was greatest when host larvae were reared on *S. alba* (61.23 ± 0.95), and this significantly exceeded that for hosts reared on *B. napus* cvs. Q2, Liberty and Conquest, *B. carinata* and *B. rapa* but not for hosts reared on *B. juncea* and *B. oleracea* (Figure 6-I).

6.3.2 Effects of Host Plant Genotypes on Life History Traits of *D. insulare*

Host plant genotype on which *P. xylostella* larvae were reared significantly affected developmental times from egg to pre-pupa, pre-pupa to pupa, pupa to adult and egg to adult for both females ($F = 50.92$; df = 7; $P < 0.0001$, $F = 14.67$; df = 7; $P < 0.0001$, $F = 5.19$; df = 7; $P < 0.0001$ and $F = 37.42$; df = 7; $P < 0.0001$ respectively) and males ($F = 46.52$; df = 7; $P < 0.0001$, $F = 5.49$; df = 7; $P < 0.0001$, $F = 4.15$; df = 7; $P = 0.0004$ and $F = 30.54$; df = 7; $P < 0.0001$ respectively) (Table 6-II). Foliage consumption by *P. xylostella* larvae varied significantly when parasitized by female and male larvae of *D. insulare* ($F = 6.03$; df = 7; $P < 0.0001$ and $F = 4.61$; df = 7; $P = 0.0001$ respectively), and pupal weights differed significantly for females and males ($F = 2.89$; df = 7; $P = 0.0076$ and $F = 3.06$; df = 7; $P = 0.0050$ respectively) (Table 6-III). Silk weight of female *D. insulare* specimens was significantly affected by plant genotype on which their hosts were reared ($F = 5.56$; df = 7; $P < 0.0001$) but not of males ($F = 2.02$; df = 7; $P = 0.0569$) (Table 6-IV). Host plant genotype significantly affected adult body weight and longevity (without food) of females ($F = 4.86$; df = 7; $P < 0.0001$ and $F = 7.83$; df = 7; $P < 0.0001$ respectively) and males ($F = 15.91$; df = 7; $P < 0.0001$ and $F = 28.88$; df = 7; $P < 0.0001$ respectively). Forewing and hindwing areas varied significantly for females ($F = 25.73$; df = 7; $P < 0.0001$ and $F = 29.15$; df = 7; $P < 0.0001$ respectively) and males ($F = 22.94$; df = 7; $P < 0.0001$ and $F = 16.80$; df = 7; $P < 0.0001$ respectively) (Table 6-V).

Development of female and male *D. insulare* from egg to pre-pupa was fastest when hosts were reared on *B. juncea* and slowest on *B. oleracea* (Table 6-II). Both female and male pupae developed fastest on *B. napus* cv. Liberty. *Plutella xylostella* larvae harboring female *D. insulare* consumed the largest leaf area of *B. napus* cv. Liberty whereas least foliage consumption occurred on *B. carinata* (Table 6-III). Female and male pupae were lighter in weight when their *P. xylostella* hosts were reared on *B. oleracea* and *S. alba* respectively than on other species and cultivars evaluated (Table 6-IV). Female specimens reared on *P. xylostella* hosts that consumed *S. alba* produced significantly more silk than those from hosts that consumed *B. napus* cv. Q2 and *B. oleracea*, but no significant differences were observed among those reared on other host plants tested. Silk weight for males did not differ significantly regardless of genotype on which their hosts were reared (Table 6-IV).

Heaviest females of *D. insulare* were produced on *P. xylostella* hosts reared on *B. carinata*; however, males were heaviest when reared on larvae that consumed *B. rapa* (Table 6-V). Female and male *D. insulare* reared on *P. xylostella* hosts that consumed *B. napus* cv. Q2 lived for shortest time in the absence of food (Table 6-V). Female wasps reared on larvae that fed on *B. carinata*, *B. rapa* and *B. oleracea* had the largest forewings whereas both male and female wasps reared on larvae that fed on *B. napus* cv. Q2 had the smallest forewings (Table 6-V). Females reared on *P. xylostella* larvae that consumed *B. napus* cv. Liberty and *B. rapa* developed the largest hindwings; males with largest hindwings were from larvae reared on *B. rapa*. Male wasps reared on larvae that consumed *B. napus* cv. Q2 had the smallest hindwings (Table 6-V).

For female *D. insulare* reared on *P. xylostella* hosts that consumed various host plant genotypes, significant positive correlations were found between herbivory and pupal weight ($r = 0.39$; $P < 0.0001$), herbivory and silk weight ($r = 0.33$; $P < 0.0001$), pupal weight and silk weight ($r = 0.67$; $P < 0.0001$), pupal weight and adult weight ($r = 0.38$; $P < 0.0001$), pupal weight and forewing area ($r = 0.25$; $P = 0.0005$), and forewing area and hindwing area ($r = 0.35$; $P < 0.0001$), but no correlations existed between herbivory and adult weight ($r = 0.08$; $P = 0.2888$), pupal weight and longevity ($r = 0.02$; $P = 0.7541$), pupal weight and hindwing area ($r = 0.10$; $P = 0.2278$), and adult weight and longevity ($r = 0.004$; $P = 0.9597$). For male *D. insulare*, significant positive correlations were found between herbivory and pupal weight ($r = 0.45$; $P < 0.0001$), herbivory and silk weight ($r = 0.52$; $P < 0.0001$), herbivory and adult weight ($r = 0.23$; $P = 0.0035$), pupal weight and silk weight ($r = 0.41$; $P < 0.0001$), pupal weight and adult weight ($r = 0.46$; $P < 0.0001$), pupal weight and longevity ($r = 0.27$; $P = 0.0006$), pupal weight and forewing area ($r = 0.46$; $P < 0.0001$), pupal weight and hindwing area ($r = 0.18$; $P = 0.0267$), forewing area and hindwing area ($r = 0.38$; $P < 0.0001$), and adult weight and longevity ($r = 0.19$; $P = 0.0168$).

Female and male *D. insulare* specimens exhibited significant differences in only a few life history traits when reared on the same host plant genotype (Table 6-VI). Foliage consumption by *P. xylostella* larvae containing either female or male *D. insulare* did not differ significantly when reared on *B. napus* cvs. Liberty and Conquest, *B. rapa* and *S. alba*. Female and male pupal weights did not differ on the tested plant genotypes except *B. oleracea* and *S. alba*. Male specimens produced more silk when reared on *B. napus* cv. Q2 but the reverse was true when specimens were reared on *S. alba*. Female wasps had larger forewings than their male counterparts when reared on *B. napus* cvs. Liberty and Conquest, *B. rapa*, *B. carinata* and *S. alba*. Males had larger hindwings than females when reared on *B. juncea* whereas females had larger hindwings than males when reared on *B. napus* cvs. Q2 and Liberty, *B. carinata* and *S. alba* (Table 6-VI).

6.3.3 Comparison for Foliage Consumption by Parasitized and Non-parasitized *Plutella xylostella* Larvae

Mean foliage consumption by parasitized *P. xylostella* larvae was significantly less than their non-parasitized counterparts on the tested plants except *B. napus* cv. Liberty where herbivory did not differ between parasitized and non-parasitized larvae. *Plutella xylostella* larvae parasitized with female *D. insulare* consumed 1.05-, 1.25-, 1.30-, 1.32-, 1.38-, 1.40-, 1.61- and 2.09-fold less foliage than their non-parasitized counterparts when reared on *B. napus* cv. Liberty, *B. napus* cv. Q2, *B. juncea*, *S. alba*, *B. oleracea*, *B. napus* cv. Conquest, *B. carinata* and *B. rapa* respectively (Table 6-III).

6.4 Discussion

6.4.1 Survival and Parasitism of *D. insulare*

Survival and parasitism of *D. insulare* varied considerably among the tested plant genotypes on which host larvae were reared with greatest on *S. alba* and least on *B. napus* cv. Q2. Levels of parasitism of the same insect herbivore by *D. insulare* and other parasitoid species have frequently been observed to differ among plant species/cultivars, and this is usually attributed to plant morphology, biochemical components of the plants, and other factors. For instance, *P. xylostella* parasitism by *D. insulare* was higher on excised leaves of *Sinapis arvensis* L. than on *B. napus* (Idris and Grafius, 1996). *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) parasitized 4- to 15-fold more host larvae on Chinese cabbage, *Brassica campestris* L. ssp. *pekinensis*, than on common cabbage, *Brassica oleracea* L. var. *capitata*, in laboratory experiments and these differences were associated with the volatile profiles of the tested species (Liu and Jiang, 2003). In a laboratory and greenhouse study, parasitism of *P. xylostella* larvae by each of the two parasitoids, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *C. plutellae*, differed significantly among four *Brassica* genotypes tested (*B. oleracea* var. *capitata*, *B. oleracea* var. *italica*, *B. oleracea* var. *botrytis*, and *B. campestris* ssp. *pekinensis*) (Talekar and Yang, 1991). In New Zealand, field sampling of crops of *B. oleracea* var. *capitata*, *B. oleracea* var. *botrytis* and *B. oleracea* var. *italica* indicated that levels of parasitism of *P. xylostella* by the larval parasitoid, *D. semiclausum*, and the pupal parasitoid, *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), were highest when host larvae were reared on *B. oleracea* var. *botrytis* (Beck and Cameron, 1990). Field observations in the Cameron Highlands of Malaysia suggested that *D. semiclausum* exerted more control of *P. xylostella* populations on *B. oleracea* var. *capitata* than on *B. campestris* ssp. *pekinensis* (Verkerk and Wright, 1997). Host plant resistance is an important tool when developing integrated management programs for pests of brassicaceous crops, and recommendations to growers of the most resistant genotypes for production usually consider only responses of herbivores, without consideration of possible effects on higher trophic levels. My studies emphasize the need to consider effects of genotype on both the herbivore and its natural enemies when making recommendations for field use. For example, survival of *P. xylostella* did not differ on the plant genotypes evaluated here (Sarfraz et al., 2007), but significant differences occurred among genotypes in the survival of *D. insulare* (Table 6-I).

Field studies are important, but parasitoid performance in the field is usually influenced by numerous biotic and abiotic factors, and apparent differences between plant genotypes may not necessarily be the result of intrinsic properties of plants alone. Therefore, detailed studies under controlled conditions should be undertaken to determine the effects of plants that could cascade up to the higher trophic levels (Liu and Jiang, 2003). To date, most laboratory studies have used excised leaves instead of intact plants, and *P. xylostella* larvae were exposed to parasitoids in small containers (e.g. Idris and Grafius, 1996; Okine et al., 1996; Monnerat et al., 2002). Results of such experiments should be interpreted with caution because they usually did not provide ideal conditions for investigating tritrophic interactions. On the other hand, studies that utilized intact plants determined percent parasitism either by dissecting the freshly parasitized host larvae that received parasitoid eggs or by counting the numbers of specimens that successfully developed into parasitoid pupae (e.g. Talekar and Yang, 1991; Yang et al., 1994; Okine et al., 1996; Karimzadeh et al., 2004). Some *P. xylostella* larvae are killed apparently from physical damage incurred during the act of parasitization or possibly as a result of foreign material delivered to the host during oviposition (Sarfraz, personal observation), whereas some parasitized larvae manage to successfully develop into herbivore host pupae possibly by killing the parasitoid eggs owing to their efficient immune systems (Benrey and Denno, 1997; Turlings and Benrey, 1998). I was unable to determine whether the above cited studies considered such possibilities, but the percent parasitism calculated in the present study included 'successful' parasitism, and corrected mortality.

6.4.2 Effects of Host Plant Genotypes on Life History Traits of *D. insulare*

Plant genotype on which *P. xylostella* hosts were reared significantly affected developmental times of both female and male *D. insulare*. These effects may be due to the presence or absence of specific nutrients in the host's diet, the action of allelochemicals detrimental to the parasitoid, or an interaction between nutrients and allelochemicals (Turlings and Benrey, 1998). For female specimens, slowest development from egg to pre-pupa, from pre-pupa to pupa, and from pupa to adult occurred on *B. oleracea*, *B. rapa* and *B. carinata* respectively. According to the Slow Growth-High Mortality Hypothesis, individuals feeding on larvae reared on these plant genotypes would have an extended window of vulnerability to a variety of mortality factors (Benrey and Denno, 1997). It has been previously reported that development of parasitized larvae was slower than their non-parasitized counterparts (Moreau et al., 2002), but the present findings indicated that it was strongly influenced by food plants;

larval development times did not differ between parasitized and non-parasitized individuals when reared on *B. napus* cvs. Q2 and Conquest, *B. juncea* and *B. carinata*.

Digital analysis indicated that *P. xylostella* larvae parasitized with female *D. insulare* consumed the largest and smallest leaf areas when reared on *B. napus* cv. Liberty and *B. carinata* respectively; males exhibited a different trend of foliage consumption. Parasitized larvae that consumed more food subsequently developed into heavier pupae and produced heavier silk cocoons. *Plutella xylostella* larvae parasitized by *D. insulare* consumed significantly less foliage than their non-parasitized counterparts on the eight Brassicaceae evaluated, suggesting that this parasitoid provided an indirect benefit to the plants. Okine et al. (1996) reported that *P. xylostella* larvae parasitized by *D. insulare* consumed less collard leaf area than unparasitized larvae. Contrary to the present findings, *P. xylostella* larvae parasitized by *C. plutellae* consumed significantly more food than non-parasitized individuals (Shi et al., 2002). Many solitary koinobiont parasitoids are known to regulate host growth by reducing total food consumption, with parasitized hosts attaining only a fraction of the size of non-parasitized hosts. For instance, *P. xylostella* larvae parasitized by an unknown species of *Diadegma* consumed ca. 35% less leaf surface of cabbage compared with non-parasitized larvae (Monnerat et al., 2002). In addition, *P. xylostella* larvae parasitized by *D. semiclausum* consumed 1.07- to 1.24-fold less cabbage foliage than their non-parasitized counterparts (Yang et al., 1994). Similarly, reduced food consumption resulting from parasitization by a solitary parasitoid has previously been reported for larvae of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) parasitized by *Lixophaga diatraeae* (Townsend) (Diptera: Tachinidae) (Brewer and King, 1978), larvae of *Ostrinia nubilalis* (Hübner)

(Lepidoptera: Pyralidae) parasitized by *L. diatraeae* (Huebner and Chiang, 1982), and for larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) parasitized by *Ophion flavidus* Brulle (Hymenoptera: Ichneumonidae) (Rohlfs and Mack, 1983).

Plant genotypes tested in this study influenced the pupal weights, silk weights, adult body weights, longevities, and sizes of forewings and hindwings of *D. insulare* when parasitized *P. xylostella* hosts were reared on various Brassicaceae. Pupal weight is a good indicator of offspring fitness as heavier pupae are known to produce larger and more fecund adults than their smaller counterparts (Barah and Sengupta, 1991). Production of more silk may provide pupae a firmer attachment to the substrate as well as greater protection from natural enemies than lesser quantities of silk. In previous studies, parasitoids emerging from hosts reared on different host plants differed significantly in their body sizes. For example, individuals of the pupal parasitoid *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) emerging from hosts reared on leaves of red oak were larger than from hosts on other host plants (Greenblatt and Barbosa, 1981). Body size is an important fitness correlate because it often affects reproductive success through variations in fecundity, longevity, searching efficiency, and host handling strategies (Charnov and Skinner, 1984; Visser, 1994; Kazmer and Luck, 1995; Ebon et al., 2000). Reduced longevity when food is limiting could impair reproductive success if females are not in the habitats of host herbivores. Reduced wing areas could also affect fitness by affecting dispersal and host searching efficiency of parasitoids.

Pre-imaginal developmental times of *P. xylostella*, pupal and adult weights, forewing areas, and adult longevities in the absence of food varied with host plant genotype (Chapter 3), and this study shows that several fitness correlates of the *D.*

insulare were also linked with the plant genotype on which herbivore hosts were reared. Such thorough analyses of tritrophic relationships are rarely incorporated in the integrated management of pests, yet this study shows that without understanding the complexities and interactions that occur in these systems, important management aspects can be missed.

Table 6-I. Mean percent survival (± S.E.) of *Diadegma insulare* from egg to pupa and from pupa to adult, when parasitized host *Plutella xylostella* larvae (n = 10) were reared on intact plants of different Brassicaceae.

Host Plant Genotype	Survival (%)	
	Egg to Pupa (%)	Pupa to Adult (%)
Brassica napus cv. Q2	42.67 ^{c}	88.00 ^{ab}
	(6.24)	(8.00)
Brassica napus cv. Liberty	45.61 ^{bc}	77.00 ^{ab}
	(6.48)	(9.17)
Brassica napus cv. Conquest	54.00 ^{abc}	68.76 ^{ab}
	(3.45)	(6.87)
Brassica juncea	62.06 ^{abc}	81.02 ^{ab}
	(3.80)	(4.17)
Brassica rapa	53.33 ^{abc}	65.67 ^{ab}
	(5.16)	(5.82)
Brassica carinata	48.44 ^{abc}	55.26 ^{b}
	(4.02)	(3.66)
Brassica oleracea	66.33 ^{ab}	88.45 ^{a}
	(2.66)	(2.71)
Sinapis alba	70.89 ^{a}	85.12 ^{ab}
	(3.07)	(2.90)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 6-II. Mean (± S.E.) developmental times of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized host *Plutella xylostella* larvae were reared on leaf tissue of various Brassicaceae.

Host Plant Genotype	Egg to Pre-pupa (days)		Pre-pupa to Pupa (days)		Pupa to adult (days)		Egg to adult (days)	
	Female	Male	Female	Male	Female	Male	Female	Male
Brassica napus cv. Q2	6.40 ^{c}	6.00 ^{c}	1.20 ^{d}	1.50 ^{b}	8.80 ^{abc}	9.20 ^{a}	16.40 ^{b}	16.70 ^{b}
	(0.18)	(0.10)	(0.09)	(0.11)	(0.22)	(0.17)	(0.18)	(0.31)
Brassica napus cv. Liberty	5.60 ^{d}	5.80 ^{cd}	1.60 ^{bcd}	1.50 ^{b}	8.20 ^{c}	8.30 ^{b}	15.40 ^{c}	15.60 ^{c}
	(0.15)	(0.20)	(0.11)	(0.11)	(0.09)	(0.11)	(0.15)	(0.21)
Brassica napus cv. Conquest	5.80 ^{d}	5.80 ^{cd}	1.20 ^{d}	1.20 ^{b}	8.50 ^{bc}	8.70 ^{ab}	15.50 ^{c}	15.70 ^{c}
	(0.09)	(0.09)	(0.09)	(0.09)	(0.18)	(0.18)	(0.18)	(0.18)
Brassica juncea	5.40 ^{d}	5.30 ^{d}	1.70 ^{bc}	1.70 ^{ab}	9.00 ^{ab}	9.00 ^{a}	16.10 ^{bc}	16.00 ^{bc}
	(0.15)	(0.18)	(0.15)	(0.18)	(0.07)	(0.07)	(0.14)	(0.07)
Brassica rapa	7.00 ^{b}	7.60 ^{ab}	2.40 ^{a}	1.60 ^{ab}	8.90 ^{abc}	8.90 ^{ab}	18.30 ^{a}	18.10 ^{a}
	(0.10)	(0.18)	(0.15)	(0.11)	(0.07)	(0.16)	(0.15)	(0.24)
Brassica carinata	7.00 ^{b}	7.00 ^{b}	2.00 ^{ab}	2.05 ^{a}	9.40 ^{a}	9.20 ^{a}	18.40 ^{a}	18.25 ^{a}
	(0.07)	(0.15)	(0.10)	(0.09)	(0.26)	(0.09)	(0.32)	(0.16)
Brassica oleracea	8.05 ^{a}	8.10 ^{a}	1.20 ^{d}	1.30 ^{b}	8.70 ^{abc}	8.90 ^{ab}	17.95 ^{a}	18.30 ^{a}
	(0.11)	(0.16)	(0.09)	(0.11)	(0.23)	(0.22)	(0.34)	(0.31)
Sinapis alba	5.60 ^{d}	5.70 ^{cd}	1.40 ^{cd}	1.30 ^{b}	9.20 ^{ab}	9.00 ^{a}	16.20 ^{bc}	16.00 ^{bc}
	(0.11)	(0.11)	(0.11)	(0.11)	(0.14)	(0.07)	(0.14)	(0.07)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$)

Table 6-III. Mean (± S.E.) foliage consumption by *Plutella xylostella* larvae parasitized by *Diadegma insulare* when reared on leaf tissue of various Brassicaceae (see Chapter 3 for foliage consumption by non-parasitized *Plutella xylostella* larvae).

Host Plant Genotype	Foliage consumed (cm^2) by parasitized larvae		Non-parasitized vs. Parasitized (*t*-values)
	Female (n = 20)	Male (n = 20)	
Brassica napus cv.	2.41 ^{ab}	1.82 ^{ab}	3.49**
Q2	(0.19)	(0.19)	
Brassica napus cv.	2.81 ^{a}	2.23 ^{a}	-0.42 ^{ns}
Liberty	(0.25)	(0.19)	
Brassica napus cv.	1.89 ^{bc}	1.84 ^{ab}	4.89***
Conquest	(0.07)	(0.08)	
Brassica juncea	1.96 ^{bc}	1.49 ^{b}	5.01***
	(0.14)	(0.09)	
Brassica rapa	1.79 ^{bc}	1.64 ^{b}	7.35***
	(0.21)	(0.12)	
Brassica carinata	1.63 ^{c}	2.33 ^{a}	4.11***
	(0.18)	(0.13)	
Brassica oleracea	2.19 ^{abc}	1.85 ^{ab}	7.23***
	(0.05)	(0.14)	
Sinapis alba	1.80 ^{bc}	2.01 ^{ab}	2.37*
	(0.12)	(0.11)	

Means in a column followed by the same letter do not differ significantly ($P = 0.05$); ^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

Table 6-IV. Mean (± S.E.) pupal weights and silk weights of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized *Plutella xylostella* host larvae were reared on leaf tissue of various Brassicaceae.

Host Plant Genotype	Pupal weight (mg)		Silk weight (mg)	
	Female	Male	Female	Male
Brassica napus cv. Q2	4.08^{ab}	3.94^{ab}	0.543^{c}	0.719^{a}
	(0.12)	(0.15)	(0.025)	(0.039)
Brassica napus cv. Liberty	4.24^{ab}	3.99^{ab}	0.679^{ab}	0.600^{a}
	(0.20)	(0.13)	(0.044)	(0.028)
Brassica napus cv. Conquest	3.91^{ab}	3.89^{b}	0.646^{abc}	0.610^{a}
	(0.10)	(0.09)	(0.015)	(0.020)
Brassica juncea	3.99^{ab}	3.86^{b}	0.628^{abc}	0.619^{a}
	(0.06)	(0.07)	(0.023)	(0.028)
Brassica rapa	4.36^{a}	4.40^{a}	0.711^{a}	0.612^{a}
	(0.06)	(0.12)	(0.010)	(0.046)
Brassica carinata	4.22^{ab}	4.10^{ab}	0.636^{abc}	0.694^{a}
	(0.10)	(0.10)	(0.028)	(0.027)
Brassica oleracea	3.82^{b}	4.03^{ab}	0.600^{bc}	0.625^{a}
	(0.06)	(0.07)	(0.009)	(0.015)
Sinapis alba	4.12^{ab}	3.81^{b}	0.713^{a}	0.622^{a}
	(0.05)	(0.10)	(0.020)	(0.020)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 6-V. Mean (± S.E.) adult body weights, longevities, and forewing and hindwing areas of female (n = 20) and male (n = 20) *Diadegma insulare* when parasitized *Plutella xylostella* hosts were reared as larvae on different Brassicaceae.

Host Plant Genotype	Adult body weight (mg)		Longevity without food (days)		Forewing area (cm^2)		Hindwing area (cm^2)	
	Female	Male	Female	Male	Female	Male	Female	Male
Brassica napus cv. Q2	1.26 ^{abc}	0.93 ^{c}	2.00 ^{c}	1.40 ^{d}	0.048 ^{c}	0.044 ^{e}	0.020 ^{de}	0.017 ^{e}
	(0.09)	(0.02)	(0.21)	(0.11)	(0.002)	(0.001)	(0.000)	(0.001)
Brassica napus cv. Liberty	1.35 ^{abc}	1.03 ^{bc}	2.40 ^{bc}	2.30 ^{c}	0.054 ^{b}	0.048 ^{cde}	0.029 ^{a}	0.023 ^{bcd}
	(0.11)	(0.01)	(0.11)	(0.11)	(0.002)	(0.001)	(0.001)	(0.000)
Brassica napus cv. Conquest	1.49 ^{a}	1.39 ^{a}	2.70 ^{ab}	2.30 ^{c}	0.053 ^{b}	0.047 ^{de}	0.022 ^{cd}	0.024 ^{bc}
	(0.08)	(0.06)	(0.11)	(0.11)	(0.001)	(0.001)	(0.001)	(0.001)
Brassica juncea	1.03 ^{c}	1.33 ^{ab}	2.80 ^{ab}	2.80 ^{ab}	0.053 ^{b}	0.052 ^{bc}	0.019 ^{e}	0.021 ^{cd}
	(0.09)	(0.05)	(0.09)	(0.09)	(0.001)	(0.000)	(0.001)	(0.000)
Brassica rapa	1.45 ^{ab}	1.61 ^{a}	3.10 ^{a}	3.20 ^{a}	0.063 ^{a}	0.058 ^{a}	0.029 ^{a}	0.028 ^{a}
	(0.10)	(0.10)	(0.07)	(0.09)	(0.000)	(0.001)	(0.001)	(0.001)
Brassica carinata	1.53 ^{a}	1.58 ^{a}	2.60 ^{ab}	2.80 ^{ab}	0.063 ^{a}	0.057 ^{ab}	0.027 ^{ab}	0.024 ^{bc}
	(0.03)	(0.05)	(0.11)	(0.09)	(0.001)	(0.002)	(0.001)	(0.001)
Brassica oleracea	1.07 ^{bc}	1.58 ^{a}	2.90 ^{ab}	2.90 ^{ab}	0.061 ^{a}	0.060 ^{a}	0.025 ^{bc}	0.026 ^{ab}
	(0.13)	(0.11)	(0.07)	(0.09)	(0.000)	(0.001)	(0.000)	(0.001)
Sinapis alba	1.04 ^{bc}	0.98 ^{c}	2.80 ^{ab}	2.60 ^{bc}	0.056 ^{b}	0.050 ^{cd}	0.022 ^{cd}	0.020 ^{de}
	(0.12)	(0.09)	(0.14)	(0.11)	(0.000)	(0.001)	(0.001)	(0.001)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 6-VI. Pair-wise comparison (*t*- values) between female (n = 20) and male (n = 20) *Diadegma insulare* for some key life history parameters when parasitized host *Plutella xylostella* larvae reared on the same host plant genotype.

Biological Parameters	*B. napus* Q2	*B. napus* Liberty	*B. napus* Conquest	*B. juncea*	*B. rapa*	*B. carinata*	*B. oleracea*	*S. alba*
Dev. time (egg to pre-pupa)	1.90 ns	-0.80 ns	0.00 ns	0.43 ns	-2.85**	0.00 ns	-0.25 ns	-0.65 ns
Dev. time (pre-pupa to pupa)	-2.04*	0.62 ns	0.00 ns	0.00 ns	4.23***	-0.37 ns	-0.72 ns	0.65 ns
Dev. time (pupa to adult)	-1.41 ns	-0.72 ns	-0.78 ns	0.00 ns	0.00 ns	0.74 ns	-0.63 ns	1.29 ns
Dev. time (egg to adult)	-0.84 ns	-0.77 ns	-0.78 ns	0.62 ns	0.71 ns	0.42 ns	-0.76 ns	1.29 ns
Herbivory (per larva)	2.59*	1.88 ns	0.50 ns	2.86**	0.60 ns	-3.23**	2.28*	-1.27 ns
Pupal weight	0.74 ns	1.03 ns	0.15 ns	1.42 ns	-0.29 ns	0.88 ns	-2.43*	2.85**
Silk weight	-3.80***	1.51 ns	1.40 ns	0.26 ns	2.07*	-1.49 ns	-1.42 ns	3.19**
Adult body weight	3.61***	2.82**	1.05 ns	-3.06**	-1.11 ns	-0.89 ns	-2.96**	0.39 ns
Adult longevity without food	2.56*	0.65 ns	2.69**	0.00 ns	-0.87 ns	-1.38 ns	0.00 ns	1.13 ns
Forewing area	1.54 ns	2.72**	4.25***	1.80 ns	3.31**	2.80**	1.52 ns	6.29***
Hindwing area	3.86***	5.72***	-1.09 ns	-3.64***	0.94 ns	3.10**	-1.25 ns	2.18*

ns = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

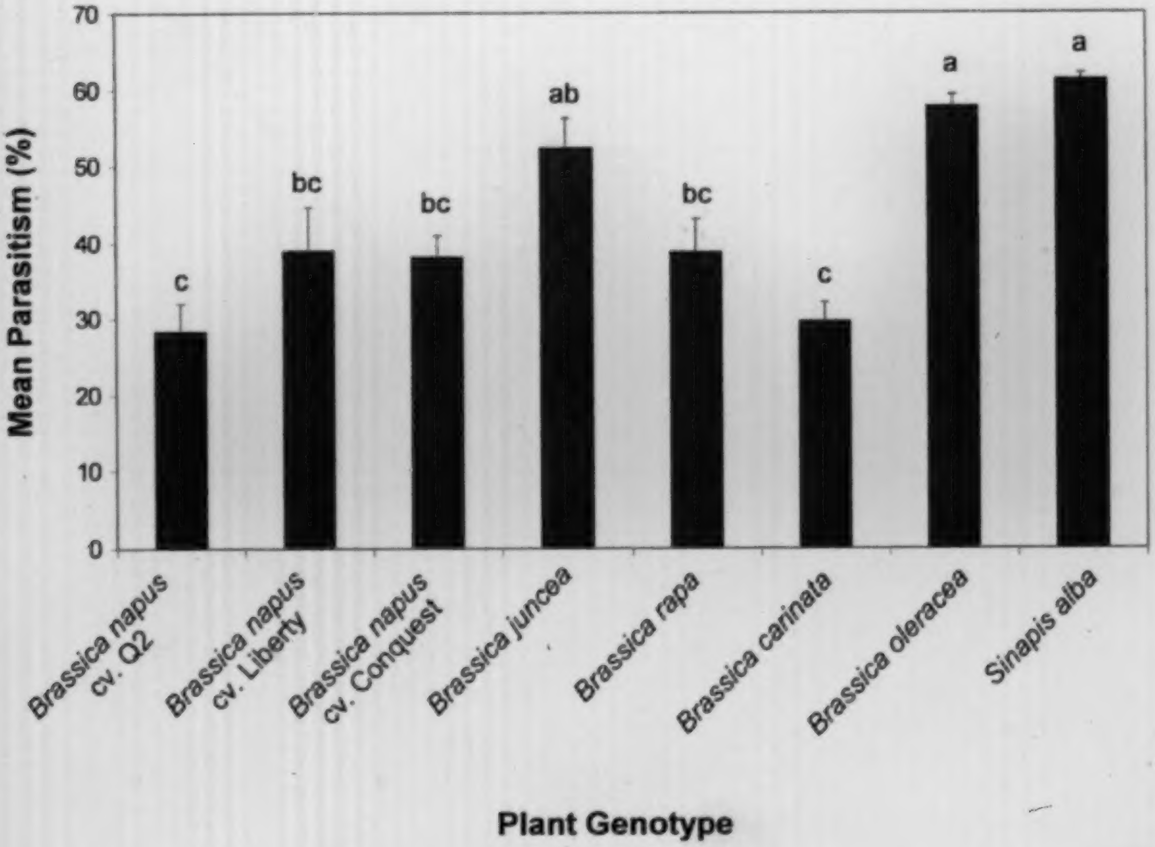

Figure 6-I. Parasitism of *Plutella xylostella* larvae by *Diadegma insulare* on intact plants in no-choice tests. Means and standard errors are presented; means with different lowercase letters are significantly different from each other (ANOVA and Tukey's mean separation, *P* = 0.05).

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Chapter 7:

Effects of Experimental Host Range Expansion by the Herbivore *Plutella xylostella* on Several Fitness Correlates of the Parasitoid *Diadegma insulare*

7.1 Introduction

Herbivorous insects routinely encounter two major challenges: feeding on a nutritionally suboptimal or sometimes even toxic resource, and the risk of being eaten (Price et al., 1980; Ode, 2006). Herbivores can occasionally expand their host ranges by incorporating new food plants into their diets. Cases of host range expansion by herbivores include novel plants from the same genus or family (Hsiao, 1978; Tabashnik 1983; Singer et al., 1993; Fraser and Lawton, 1994; Camara, 1997), and from a different family (Phillips and Barnes, 1975; Bowers et al., 1992; Löhr and Gathu, 2002). For a novel plant to be incorporated into the diet, the insect herbivore must accept that plant as food and have sufficient offspring fitness on it (Rausher, 1982; Thompson 1988; Bowers et al., 1992). The use of a new host plant may increase food availability, and change the vulnerability of the opportunist herbivore to its natural enemies (Price et al., 1980; Scriber and Slansky, 1981; Tabashnik, 1983; Bernays and Graham, 1988; Bowers et al., 1992; Camara, 1997). One advantage of the acquisition of novel plants by herbivores is that this can provide an enemy-free space owing to lack of adaptation of specialist parasitoids to their herbivorous hosts on new food plants (Jeffries and Lawton, 1984; Gratton and Welter, 1999; Gross et al., 2004; Mulatu et al., 2004). The effects of an expanded host range can be intricate, extending to several trophic levels. For instance, plants can negatively affect parasitoid fitness directly when the developing parasitoid encounters the plant allelochemicals inside its herbivore host and/or indirectly when the parasitoid is stressed due to compromised herbivore host quality from being reared on a suboptimal plant (Price et al., 1980; Ode, 2006). The relationship between plant chemistry and herbivore host location behavior of parasitoids is well documented (e.g., Turlings et al., 1995; Potting et al., 1999; Ohara et al., 2003), but studies elucidating the effects of host and non-host plants on parasitoid fitness are rare.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered a specialist on Brassicaceae (Talekar and Shelton, 1993; Muhamad et al., 1994; Idris and Grafius, 1996; Sarfraz et al., 2006). Brassicaceae comprise a diverse group of cultivated and wild plants characterized by secondary plant compounds, the glucosinolates (Kjaer, 1974; Mithen, 1992). *Plutella xylostella* relies on some of the glucosinolates for host location, oviposition and feeding stimulation (Thorsteinson, 1953; Gupta and Thorsteinson, 1960a, b). *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) is a solitary, host-specific larval endoparasitoid and is an important biological control agent of *P. xylostella* (Harcourt, 1960; Fitton and Walker, 1992; Sarfraz et al., 2005). It parasitizes all four larval instars of *P. xylostella* and kills and emerges from the pre-pupal stage of its host; one host larva supports only one parasitoid larva (Harcourt, 1960).

Canola, *Brassica napus* L. (Brassicaceae), is a widely cultivated oilseed crop in western Canada and elsewhere. Flixweed, *Descurainia sophia* (L.) Webb ex Prantl, is a winter annual or biennial wild Brassicaceae and is commonly found in canola fields and fallow lands (Morishita, 1991; Mitich, 1996). The spider flower, *Cleome hassleriana*

Chodat (Capparaceae), is an ornamental herb in North America (Foster, 2001) and it readily escapes gardens to invade roadsides and the shores of rivers and lakes (Anonymous, 2006). The garden nasturtium, *Tropaeolum majus* L. (Tropaeolaceae), originated in South America but is now grown worldwide as an ornamental plant (Stephens, 2003) and often occurs as a weed on roadsides and riverbanks. The glucosinolates, glucocapparin (aliphatic allylic glucosinolate) and glucotropaeolin (aromatic glucosinolate) predominate in *C. hassleriana* and *T. majus*, respectively (Kjaer, 1974; Renwick and Lopez, 1999).

This study was designed to provide detailed insights into tritrophic interactions focusing on several fitness correlates of both male and female *D. insulare* when its *P. xylostella* host larvae were reared on two brassicaceous (*B. napus* and *D. sophia*) and two non-brassicaceous species (*C. hassleriana* and *T. majus*). *Cleome hassleriana* and *T. majus* can occur sympatrically with wild and cultivated Brassicaceae, and so could potentially serve to harbor *P. xylostella* populations, perhaps providing bridge hosts until crop plants are available. Recent research demonstrated that *P. xylostella* performance was equivalent on selected Brassicaceae and non-Brassicaceae (Sarfraz et al., 2007b). I therefore used the same plant genetic material to investigate the effects of experimental host expansion by *P. xylostella* on the third trophic level. Several key life history parameters directly related to parasitoid population dynamics (e.g., parasitism, survival, developmental time, larval herbivory, pupal weight, silk weight, adult body weight, longevity without food, forewing area, and hindwing area) were investigated.

7.2 Materials and Methods

7.2.1 Insects and Plants

The laboratory colonies of *P. xylostella* and *D. insulare* were maintained on potted *B. napus* plants at 22±0.5°C with 16h L: 8h D under growth chamber conditions. Moths and wasps collected from different fields in Alberta (Canada) were added to the culture every summer to maintain genetic diversity.

Brassica napus 'Q2', *D. sophia*, *C. hassleriana* 'Cherry Queen' and *T. majus* 'Golden Gleam' were grown under greenhouse conditions. Plants were grown individually in 15.2-cm-diameter pots using Metromix-220 (W. R. Grace & Co., Ajax, Ontario, Canada) as a potting medium fertilized with 20: 20: 20 (nitrogen: phosphorous: potassium) at 0.5 g pot^{-1} when plants were two to four weeks old. Depending on the growth habits of tested plant species, seeding dates were adjusted accordingly. Five- to seven-week-old plants were used for all experiments; i.e., fast-growing species were used when plants were five weeks old while slower-growing species were six to seven weeks old.

7.2.2 Intact Plant Study: Survival and Parasitism

Survival from egg to pupa was assessed in screened cages (40 x 40 x 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 80 cages with 20 plants from each species. All plants were infested with first-instar *P. xylostella* larvae at 10 larvae per plant by holding neonates carefully from their silk to avoid handling damage. Larvae were observed daily until they molted to second instars;

neonates had no prior feeding experience. Ten plants of each species received two female and two male wasps (≤ 3 days old) whereas the remaining ten plants served as controls (see Sarfraz et al., 2007a); wasps were allowed to parasitize *P. xylostella* larvae for 24 h and were then removed from the cages. Plants were observed every 48 h and the numbers of surviving larvae were recorded, but daily observations were made when pupation began. Pupae were harvested, weighed within 24 h of pupation and kept individually in labeled transparent plastic cups until adult emergence.

It was assumed that mortality of *P. xylostella* larvae other than control mortality was caused by parasitoids and the percent parasitism was calculated using the following equation:

$$\text{Parasitism (\%)} = [(P_{di} \div L_t) \times 100] + M_c$$

where P_{di} = numbers of *D. insulare* pupae that developed, L_t = total numbers of *P. xylostella* larvae introduced to each cage, and M_c = percent corrected mortality determined by Schneider-Orelli's formula (Schneider-Orelli, 1947).

7.2.3 Leaf Tissue Study: Pre-imaginal and Imaginal Parameters

This study was conducted in controlled environmental conditions in a growth chamber (22±0.5°C with 16h L: 8h D). Excised leaves were placed on moist filter papers (9-cm diameter) in plastic containers covered with transparent ventilated lids. For each plant species, 100 to 200 second-instar larvae (≤ 1 day old) taken from the laboratory colony were parasitized (each larva was offered to female wasps individually and observed until parasitized) and introduced into individual plastic containers; a total of 600 larvae were used (one larva per container). Larvae were provided with fresh leaf tissue every 24 h until pupation. Developmental times from egg to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 h of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were weighed using a Sartorius Supermicro® scale (Sartorius Inc., Edgewood, NY, USA). Adults were sexed and used in the longevity (without food), body weight, and forewing and hindwing area experiments.

To quantify levels of larval feeding, all leaves damaged by parasitized *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA). Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory as described by Sarfraz et al. (2007a). Leaves of *D. sophia* were too small to be scanned. In this case, an additional set of containers with uninfested foliage (control) was used in addition to containers with parasitized *P. xylostella* larvae infesting foliage (treatment). Foliage was changed daily; fresh leaves were weighed before placing them in the containers, and old leaves were weighed as soon as they were removed from the containers. Herbivory was quantified using the foliage weight losses in both treatment and control containers.

Twenty females and 20 males of *D. insulare* reared from each plant species were used to determine their longevities without food. Specimens were placed in individual plastic containers and examined daily for survival or mortality. Wasps were weighed within 24 h of their death. Forewings and hindwings were carefully removed, glued onto a paper, scanned using a desktop scanner (Umax Powerlook 2100XL Flatbed Scanner, UMAX Technologies Inc., Dallas, TX, USA), and their areas were measured using Image J as described by Sarfraz et al. (2007a).

7.2.4 Statistical Analyses

Transformations ($(x+0.5)^{0.5}$, $\ln(x+1)$) were used as necessary to achieve normality and homoscedasticity in data before analysis (Steel et al., 1997), but untransformed means are presented graphically and in tables. Analyses of variance (ANOVA) (PROC GLM) for a completely randomized design were performed to test the differences between treatments, and means were compared at the 5% level of significance using Tukey's studentized range test (Littell et al., 2002; SAS Institute, 2004). Data on larval herbivory for *D. sophia* were not included in ANOVA owing to measurement units different from other tested plant species. Correlations (PROC CORR) were determined between pupal weight and silk weight, pupal weight and adult weight, pupal weight and longevity without food, adult weight and longevity, pupal weight and forewing area, and forewing area and hindwing area for both female and male specimens. T-tests (PROC TTEST) were performed for pair-wise comparisons between female and male *D. insulare* for their developmental times, larval herbivory, pupal weights, silk weights, adult body weights, longevity (without food), forewing and hindwing areas when they were reared on the same plant species. PROC TTEST was performed for pair-wise comparisons between parasitized and non-parasitized *P. xylostella* larvae for foliage consumption on each plant species.

7.3 Results

7.3.1 Survival and Parasitism

Plant species on which *P. xylostella* larvae were reared had no effect on survival of *D. insulare* from egg to pupa ($F_{3,27} = 2.66$; $P = 0.0680$), and from pupa to adult ($F_{3,27} = 2.09$; $P = 0.1255$) (Table 7-I).

Plant species significantly affected mean percent parasitism ($F_{3,27} = 3.84$; $P = 0.0207$). When host larvae were reared on *T. majus*, percent parasitism (42.60 ± 3.70) significantly exceeded that for hosts reared on *D. sophia* but not for hosts reared on *C. hassleriana* and *B. napus* (Figure 7-I).

7.3.2 Effects of Host Plant Genotypes on Life History Traits of *D. insulare*

Host and non-host plant species on which *P. xylostella* larvae were reared significantly affected developmental times of *D. insulare* from egg to pre-pupa, pupa to adult, and egg to adult for both females ($F_{3,57} = 27.56$; $P < 0.0001$, $F_{3,57} = 16.86$; $P < 0.0001$ and $F_{3,57} = 49.11$; $P < 0.0001$ respectively) and males ($F_{3,57} = 45.47$; $P < 0.0001$, $F_{3,57} = 22.47$; $P < 0.0001$ and $F_{3,57} = 9.17$; $P < 0.0001$ respectively). Pre-pupal development time of *D. insulare* differed significantly for females ($F_{3,57} = 41.89$; $P < 0.0001$) but not for males ($F_{3,57} = 2.53$; $P = 0.0664$) when reared on host larvae that fed on the tested plant species (Table 7-II). Foliage consumption by *P. xylostella* larvae varied significantly when parasitized by female larvae of *D. insulare* ($F_{3,57} = 10.74$; $P = 0.0002$) but not for male larvae ($F_{3,57} = 0.93$; $P = 0.4017$) (Table 7-III). Pupal weights differed significantly for females and males ($F_{3,57} = 31.43$; $P < 0.0001$ and $F_{3,57} = 18.47$; $P < 0.0001$ respectively). Silk weight of female and male *D. insulare* specimens was significantly affected by plant species on which their hosts were reared ($F_{3,57} = 24.15$; $P < 0.0001$ and $F_{3,57} = 33.48$; $P < 0.0001$ respectively) (Table 7-IV). Host plant genotype significantly affected adult body weight and longevity (without food) of females ($F_{3,57} = 60.47$; $P < 0.0001$ and $F_{3,57} = 11.05$; $P < 0.0001$ respectively) and males ($F_{3,57} = 31.44$; $P < 0.0001$ and $F_{3,57} = 48.17$; $P < 0.0001$ respectively) (Table 7-V). Forewing and hindwing areas varied significantly for females ($F_{3,57} = 4.48$; $P = 0.0068$ and $F_{3,57} = 22.26$; $P < 0.0001$ respectively) and males ($F_{3,57} = 18.27$; $P < 0.0001$ and $F_{3,57} = 27.88$; $P < 0.0001$ respectively) (Table 7-V).

Female and male development of *D. insulare* from egg to pre-pupa was significantly faster when *P. xylostella* hosts were reared on *B. napus* than on any other host plant species (Table 7-II). Female pupae developed fastest on *C. hassleriana* whereas male pupal development was fastest on *D. sophia*. For both female and male specimens, egg to adult development was fastest on *B. napus* and approximately 2 d slower on *T. majus* (Table 7-II).

Plutella xylostella larvae parasitized with female *D. insulare* larvae consumed significantly greater leaf areas of *B. napus* than of *T. majus*, whereas larvae parasitized with males consumed similar leaf areas regardless of host plant species (Table 7-III). *Plutella xylostella* larvae parasitized by females of *D. insulare* consumed significantly less foliage of *B. napus*, *C. hassleriana* and *T. majus* than non-parasitized larvae, but significantly more foliage of *D. sophia* (Table 7-III). However, *P. xylostella* larvae parasitized by males of *D. insulare* consumed similar quantities of leaf tissue of *B. napus*, *C. hassleriana* and *T. majus*, although more foliage of *D. sophia* was consumed by larvae parasitized with *D. insulare* males than by non-parasitized larvae (Table 7-III).

Female pupae were lighter in weight when their *P. xylostella* hosts were reared on *D. sophia* and *C. hassleriana* than on *B. napus* and *T. majus* (Table 7-IV). Male pupae were lightest when larvae were reared on *C. hassleriana* and heaviest when reared on *D. sophia*. Both female and male specimens reared on *P. xylostella* hosts that consumed *T. majus* produced significantly more silk than those from hosts that consumed *C. hassleriana* and *D. sophia*, but non-significant differences were observed between those reared on *B. napus* and *T. majus* (Table 7-IV).

Heaviest females of *D. insulare* were produced on *P. xylostella* hosts reared on *T. majus*; however, males were heaviest when reared on larvae that consumed *D. sophia* and *T. majus* (Table 7-V). Female and male specimens reared on *P. xylostella* hosts that consumed *B. napus* lived for shortest time in the absence of food. Females reared on *P. xylostella* larvae that consumed *C. hassleriana* developed the largest forewings; males with largest forewings were from larvae reared on *D. sophia*. Female wasps reared on larvae that consumed *B. napus* had the smallest hindwings whereas male wasps reared on larvae that consumed *B. napus* and *T. majus* had the smallest hindwings (Table 7-V).

For female specimens reared on *P. xylostella* hosts that consumed various plant species, significant correlations were found between pupal weight and silk weight (r = 0.35; P = 0.0015), pupal weight and adult weight (r = 0.61; P < 0.0001), and forewing area and hindwing area (r = 0.48; P < 0.0001), but no correlations existed between pupal weight and longevity (r = -0.03; P = 0.7747), pupal weight and forewing area (r = -0.11; P = 0.3404), and adult weight and longevity (r = -0.11; P = 0.3264). For males, significant correlations were found between pupal weight and silk weight (r = 0.40; P = 0.0003), pupal weight and adult weight (r = 0.80; P < 0.0001), pupal weight and longevity ($r = 0.23$; $P = 0.0400$), pupal weight and forewing area ($r = 0.45$; $P < 0.0001$), forewing area and hindwing area ($r = 0.69$; $P < 0.0001$), and adult weight and longevity ($r = 0.45$; $P < 0.0001$).

Female and male specimens exhibited significant differences in certain life history parameters when reared on the same host plant genotype (Table 7-VI). Foliage consumption by *P. xylostella* larvae containing female *D. insulare* was significantly higher than for their male counterparts when reared on *B. napus* and *C. hassleriana* but the reverse was true when specimens were reared on *D. sophia* and *T. majus*. Female and male pupal weights did not differ on the tested plant genotypes except for *D. sophia*. Male specimens produced significantly more silk than females when reared on *B. napus*, *D. sophia* and *T. majus*. Female wasps had smaller forewings than their male counterparts when reared on *D. sophia*. Females had larger hindwings than males when reared on *B. napus* and *T. majus* whereas females and males had similar hindwing areas when reared on *C. hassleriana* (Table 7-VI).

7.4 Discussion

Parasitism by *D. insulare* varied considerably among the tested host and non-host plant species on which *P. xylostella* larvae were reared. Most parasitism occurred on *T. majus* and least on *D. sophia* whereas parasitism level was statistically similar on *B. napus* and *C. hassleriana*. Levels of parasitism of *P. xylostella* by other parasitoid species have frequently been reported to differ among plant genotypes within the same family. For instance, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) parasitized 4- to 15-fold more host larvae on Chinese cabbage, *Brassica campestris* L. ssp. *pekinensis*, than on common cabbage, *Brassica oleracea* L. var. *capitata*, in laboratory experiments (Liu and Jiang, 2003). However, this is the first study of its kind to indicate that parasitism by *D. insulare* was similar or even higher when *P. xylostella* hosts were feeding on non-brassicaceous compared with brassicaceous species. By contrast, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) parasitized 1.6-fold more *P. xylostella* larvae on *B. oleracea* var. *capitata* than on snowpeas, *Pisum sativum* L. (Fabaceae) (Rossbach et al., 2006). Similarly, parasitism of the crucifer specialist *Pieris rapae* (L.) (Lepidoptera: Pieridae) by *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) was significantly higher on *B. oleraceae* than on *T. majus* and *Cleome spinosa* L. (Benrey and Denno, 1997).

In an earlier study, *P. xylostella* accepted non-Brassicaceae (*C. hassleriana* and *T. majus*) in the presence of Brassicaceae (*B. napus* and *D. sophia*) for oviposition (Sarfraz et al., 2007b; Chapter 4), suggesting that *P. xylostella* is capable of expanding its host range by incorporating these two non-brassicaceous species into its diet. Host expansion by herbivorous insects is often linked with enemy-free space (Jeffries and Lawton, 1984; Gratton and Welter, 1999; Mulatu et al., 2004). Low oviposition by *P. xylostella* (Sarfraz et al. 2007b) and high parasitism by *D. insulare* on *C. hassleriana* and *T. majus* suggest that these two non-host plant species might not provide an enemy-free refuge for *P. xylostella*. By the same token, the combination of high *P. xylostella* oviposition and low *D. insulare* parasitism on *B. napus* leads to the conclusion that *B. napus* provides a better refugium for *P. xylostella* than the tested non-brassicaceous species. Infestations by specialist *P. xylostella* herbivores on unusual food plants may suppress the insect's immune system and thereby benefit the parasitoid. For instance, plant species significantly affected the encapsulation rate in larvae of *P. rapae* parasitized by *C. glomerata*; the percent encapsulation of parasitoid eggs and larvae was higher when herbivore hosts were reared on *B. oleracea* than on *C. spinosa* and *T. majus* (Benrey and Denno, 1997). The present study also suggests that the apparent failure of *P. xylostella* to actually extend its host range to include *T. majus* and *C. hassleriana* might have been hampered by parasitoids, such as *D. insulare*. Substantial evidence exists that restriction in host range expansion can result from differential vulnerability to natural enemies (Smiley, 1978; Gilbert, 1979; Price et al., 1980). Smiley (1978) attributed the specialization of *Heliconius melpomene* L. (Lepidoptera: Nymphalidae) on *Passiflora oerstedii* Mast. (Passifloraceae) to predator pressure despite its similar performance on four other tested *Passiflora* species.

Plant species on which *P. xylostella* hosts were reared significantly affected developmental times of both female and male *D. insulare*. Fastest development from egg to adult occurred on *B. napus* and slowest on *T. majus* and *D. sophia*. Other researchers have also reported variations in parasitoid developmental time when their hosts were reared on different plant genotypes. For example, some evidence exists that *D. semiclausum* development was slower when parasitized *P. xylostella* hosts were reared on a novel host (*P. sativum*) than on a natural host (*B. oleracea*) (Rossbach et al., 2006). Individuals of *D. insulare* feeding on larvae reared on *T. majus* and *D. sophia* may have an extended window of vulnerability to a wide array of natural enemies (Slow Growth-High Mortality Hypothesis). For example, slow-developing larvae of *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) on resistant varieties of soybean incurred greater mortality from *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) than fast-developing larvae on susceptible varieties (Bouton, 1984). Similarly, prolonged larval development of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) on tobacco plants resulted in increased mortality by the parasitoid *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) (Johnson and Gould, 1984).

Plutella xylostella larvae parasitized with female *D. insulare* consumed significantly less foliage than their non-parasitized counterparts on *B. napus*, *C. hassleriana* and *T. majus*. *Diadegma insulare* provided an indirect benefit to the plants by reducing their leaf surface areas removed due to larval herbivory, in addition to killing its herbivore. Several studies provide support for the present findings. For instance, *P. xylostella* larvae parasitized by an unknown species of *Diadegma* consumed ca. 35% less leaf surface of cabbage compared with non-parasitized larvae (Monnerat et al., 2002). Similarly, reduced food consumption was previously reported for larvae of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) parasitized by *Lixophaga diatraeae* (Townsend) (Diptera: Tachinidae) (Brewer and King, 1978), larvae of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) parasitized by *L. diatraeae* (Huebner and Chiang, 1982), and for larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) parasitized by *Ophion flavidus* Brullé (Hymenoptera: Ichneumonidae) (Rohlfs and Mack, 1983).

Plant species tested in this study influenced the pupal weights, silk weights, adult body weights, longevities, and sizes of forewings and hindwings of *D. insulare* when parasitized *P. xylostella* hosts were reared on various Brassicaceae and non-Brassicaceae. In previous studies, parasitoids emerging from hosts reared on different host plants differed significantly in their body sizes and longevities. For example, the parasitoid

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae) that developed from *Anstrepha ludens* (Loew) (Diptera: Tephritidae) hosts that fed on grapefruit were significantly larger in body sizes and lived longer than from hosts on other food sources (Ebon et al., 2000). Similarly, the pupal parasitoid *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) emerging from hosts reared on leaves of red oak (*Quercus* sp.) were larger than from hosts on other host plants (Greenblatt and Barbosa, 1981). Body size/weight is an important fitness correlate because it often affects reproductive success through variations in fecundity, dispersal, searching efficiency, and host handling strategies (Price et al., 1980; Charnov and Skinner, 1984; Visser, 1994; Kazmer and Luck, 1995; Ebon et al., 2000); smaller size resulting in lower fecundity would lower the rate of population increase.

In my earlier study, *D. sophia* was found the least suitable host plant for *P. xylostella* among the tested brassicaceous and non-brassicaceous species (Sarfraz et al., 2007b; Chapter 4). In this study, *D. insulare* performance (e.g. survival, developmental time, pupal weights, and adult body weights) was similar when parasitized hosts were reared on *D. sophia*. However, female and male *D. insulare* reared on *D. sophia* performed differently in most of the fitness correlates. For instance, female and male adult emergence did not coincide ($t = 2.29$; $P = 0.0059$) when specimens were reared on *D. sophia* and such temporal differences may adversely affect the reproductive success and population dynamics of *D. insulare*.

Table 7-I. Mean percent survival (± S.E.) of *Diadegma insulare* from egg to pupa and from pupa to adult, when parasitized host *Plutella xylostella* larvae (n = 10) were reared on intact plants of two Brassicaceae and two non- Brassicaceae.

Plant Species	% Survival	
	Egg to Pupa (%)	Pupa to Adult (%)
Brassica napus	45.78 [a]	86.67 [a]
	(5.89)	(7.10)
Descurainia sophia	44.52 [a]	60.67 [a]
	(6.25)	(8.56)
Cleome hassleriana	53.00 [a]	70.64 [a]
	(3.06)	(5.01)
Tropaeolum majus	67.31 [a]	73.85 [a]
	(5.56)	(5.40)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 7-II. Mean (± S.E.) developmental times of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized host *Plutella xylostella* larvae were reared on leaf tissue of *Brassica napus, Descurainia sophia, Cleome hassleriana* and *Tropaeolum majus.*

		Plant Species			
Biological parameters		*Brassica napus*	*Descurainia sophia*	*Cleome hassleriana*	*Tropaeolum majus*
Egg to pre-pupa (days)	Female	6.40^{b}	8.00^{a}	8.60^{a}	8.50^{a}
		(0.17)	(0.23)	(0.18)	(0.11)
	Male	6.10^{b}	8.35^{a}	8.05^{a}	7.85^{a}
		(0.10)	(0.11)	(0.15)	(0.18)
Pre-pupa to pupa (days)	Female	1.18^{b}	1.75^{a}	1.05^{b}	0.75^{c}
		(0.08)	(0.06)	(0.06)	(0.06)
	Male	1.50^{a}	1.18^{a}	1.25^{a}	1.50^{a}
		(0.11)	(0.05)	(0.10)	(0.11)
Pupa to adult (days)	Female	8.75^{b}	8.50^{bc}	8.05^{c}	9.50^{a}
		(0.22)	(0.11)	(0.09)	(0.11)
	Male	9.00^{a}	8.00^{b}	8.10^{b}	9.00^{a}
		(0.16)	(0.07)	(0.14)	(0.07)
Egg to adult (days)	Female	16.33^{c}	18.25^{ab}	17.70^{b}	18.75^{a}
		(0.17)	(0.17)	(0.16)	(0.06)
	Male	16.60^{c}	17.53^{ab}	17.40^{bc}	18.35^{a}
		(0.28)	(0.18)	(0.23)	(0.21)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 7-III. Mean (± S.E.) foliage consumption (cm^2 or g) by *Plutella xylostella* larvae when reared on leaf tissue of *Brassica napus*, *Descurainia sophia*, *Cleome hassleriana* and *Tropaeolum majus* and either parasitized or non-parasitized by *Diadegma insulare*.

Plant Species	Foliage consumed by non-parasitized larvae¶		Foliage consumed by parasitized larvae		Non-parasitized vs. Parasitized (*t*-test)	
	Female (n = 20)	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female	Male
Brassica napus	3.02 ^{a} (0.13)	2.13 ^{a} (0.16)	2.44 ^{a} (0.19)	1.83 ^{a} (0.13)	2.55*	1.49 ^{ns}
Descurainia sophia	0.08 ‡ (0.00)	0.05 ‡ (0.00)	0.21 ‡ (0.01)	0.30 ‡ (0.01)	-9.64***	-17.04***
Cleome hassleriana	2.84 ^{a} (0.12)	1.91 ^{ab} (0.10)	2.14 ^{a} (0.07)	1.65 ^{a} (0.16)	5.19***	1.40 ^{ns}
Tropaeolum majus	2.06 ^{b} (0.08)	1.64 ^{b} (0.10)	1.70 ^{b} (0.00)	1.85 ^{a} (0.04)	4.25***	-2.01*

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test; ¶ Data taken from Chapter 4; ‡ Data were not included in analysis as amounts of tissue consumed were in grams; ^{ns} = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; *** = significant at $P \leq 0.001$

Table 7-IV. Mean (± S.E.) pupal weights and silk weights of *Diadegma insulare* female (n = 20) and male (n = 20) specimens when parasitized *Plutella xylostella* host larvae were reared on leaf tissue of two Brassicaceae and two non-Brassicaceae.

Plant Species	Pupal weight (mg)		Silk weight (mg)	
	Female	Male	Female	Male
Brassica napus	4.17^{a}	3.90^{b}	0.554^{ab}	0.721^{ab}
	(0.11)	(0.14)	(0.023)	(0.038)
Descurainia sophia	3.30^{b}	4.32^{a}	0.550^{b}	0.644^{b}
	(0.09)	(0.07)	(0.001)	(0.018)
Cleome hassleriana	3.19^{b}	3.25^{c}	0.343^{c}	0.430^{c}
	(0.08)	(0.14)	(0.048)	(0.025)
Tropaeolum majus	3.95^{a}	3.95^{ab}	0.653^{a}	0.753^{a}
	(0.08)	(0.01)	(0.001)	(0.004)

Means in a column followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 7-V. Mean (± S.E.) adult body weights, longevities, and forewing and hindwing areas of female (n = 20) and male (n = 20) *Diadegma insulare* when parasitized *Plutella xylostella* hosts were reared as larvae on two Brassicaceae and two non-Brassicaceae.

Biological parameters		Plant Species *Brassica napus*	 *Descurainia sophia*	 *Cleome hassleriana*	 *Tropaeolum majus*
Adult body weight (mg)	Female	1.22^{b}	0.75^{c}	0.74^{c}	1.40^{a}
		(0.08)	(0.01)	(0.02)	(0.02)
	Male	0.93^{b}	1.38^{a}	0.70^{c}	1.30^{a}
		(0.02)	(0.05)	(0.09)	(0.02)
Longevity without food (days)	Female	2.05^{c}	2.50^{bc}	3.15^{a}	2.70^{ab}
		(0.20)	(0.11)	(0.08)	(0.15)
	Male	1.50^{c}	3.35^{a}	2.75^{b}	2.90^{b}
		(0.11)	(0.11)	(0.00)	(0.12)
Forewing area (cm^2)	Female	0.048^{ab}	0.046^{b}	0.052^{a}	0.048^{ab}
		(0.001)	(0.000)	(0.001)	(0.001)
	Male	0.045^{c}	0.057^{a}	0.051^{b}	0.046^{bc}
		(0.001)	(0.002)	(0.001)	(0.000)
Hindwing area (cm^2)	Female	0.020^{c}	0.021^{bc}	0.025^{a}	0.022^{b}
		(0.000)	(0.000)	(0.000)	(0.000)
	Male	0.018^{b}	0.025^{a}	0.024^{a}	0.018^{b}
		(0.001)	(0.000)	(0.001)	(0.001)

Means in a row followed by the same letter do not differ significantly ($P = 0.05$) using analysis of variance and Tukey's studentized range test

Table 7-VI. Pair-wise comparisons (*t*-values) between female (n = 20) and male (n = 20) *Diadegma insulare* for some key life history parameters when parasitized host *Plutella xylostella* larvae were reared on leaf tissue of *Brassica napus, Descurainia sophia, Cleome hassleriana* and *Tropaeolum majus*.

Biological parameters	Plant Species			
	Brassica napus	*Descurainia sophia*	*Cleome hassleriana*	*Tropaeolum majus*
Development time (egg to pre-pupa)	1.53 ns	-1.38 ns	2.30 ns	3.02**
Development time (pre-pupa to pupa)	-2.29*	7.25***	-1.71 ns	-5.85***
Development time (pupa to adult)	-0.93 ns	3.68***	-0.30 ns	3.68***
Development time (egg to adult)	-0.83 ns	2.29**	1.06 ns	1.85 ns
Herbivory (per larva)	2.70**	-4.34***	2.90**	-4.20***
Pupal weight	1.53 ns	-8.92***	-0.40 ns	0.00 ns
Silk weight	-3.75***	-5.27***	-1.61 ns	-23.33***
Adult body weight	3.41**	-13.23***	0.42 ns	3.08**
Adult longevity (without food)	2.40*	-5.36***	3.11**	-1.04 ns
Forewing area	1.42 ns	-6.42***	0.98 ns	1.38***
Hindwing area	3.14**	-14.14***	0.64 ns	3.90

ns = non-significant at $P > 0.05$; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

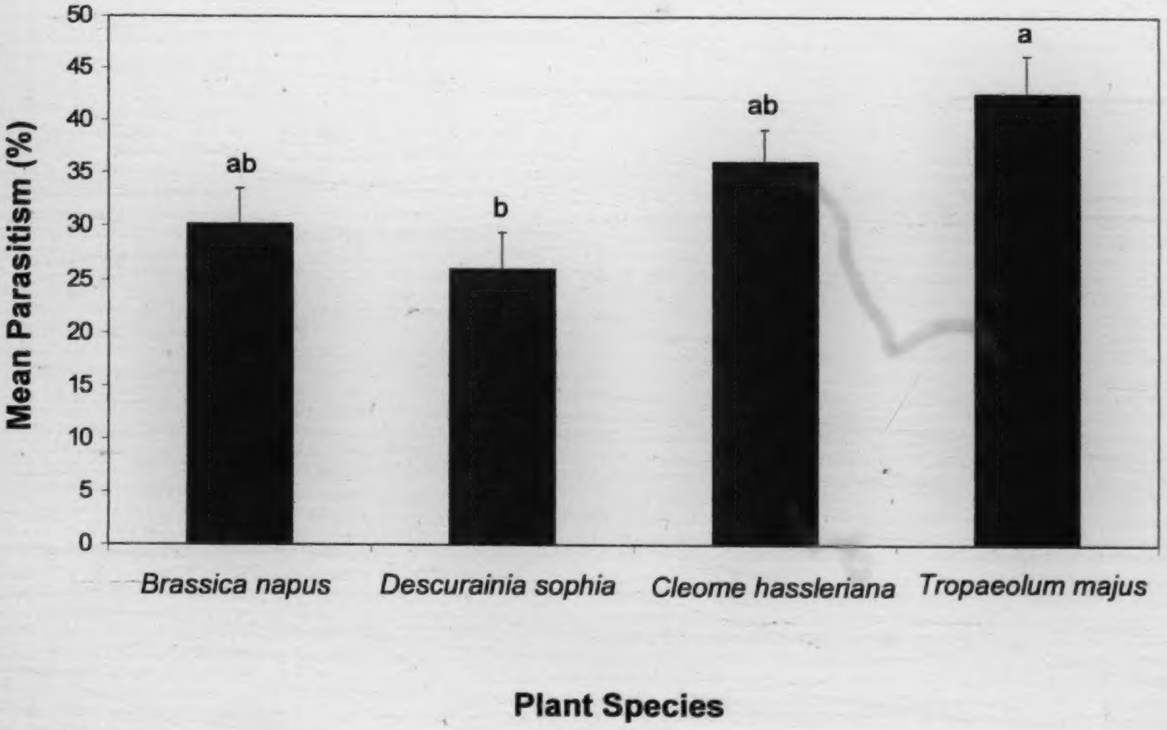

Figure 7-I. Parasitism of *Plutella xylostella* larvae (n = 10) by *Diadegma insulare* on intact plants in no-choice tests. Means and standard errors are presented; means with different lowercase letters are significantly different from each other (ANOVA and Tukey's mean separation, $P = 0.05$).

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Chapter 8:

The Spatio-temporal Distributions of *Plutella xylostella* and Its Larval Parasitoids *Diadegma insulare* and *Microplitis plutellae* in Canola: Implications for Pest Management

8.1 Introduction

Parasitoids can provide biological control of certain insect pests, but their effectiveness depends upon their synchrony in space and time with the appropriate life stage of their hosts (Murchie et al., 1999; Ferguson et al., 2000; Warner et al., 2000; Dosdall et al., 2006). Understanding the spatio-temporal distribution patterns of herbivorous pest species and their associated parasitoids in agro-ecosystems is an important prerequisite for the development of modern integrated pest management (IPM) strategies. This understanding may allow spatial and temporal targeting of insecticides to crop areas of highest pest densities, with applications timed to coincide with invasion events of the pests before arrival of natural enemies. When implemented successfully, this strategy can enhance insecticide efficiency by suppressing pest infestations while conserving natural enemies (Ferguson et al., 2000). Temporal targeting of insecticides has been applied successfully in winter canola (*Brassica napus* L.) to conserve populations of *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae), a principal parasitoid of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), through avoidance of chemical application during the main period of the parasitoid immigration into the crop (Murchie et al., 1997; Ferguson et al., 2000). In addition to improving the environmental sustainability of chemical control strategies, understanding spatio-temporal distribution patterns of a pest and its natural enemies may play a crucial role in implementing additional IPM tactics such as the 'push-pull' strategy and assigning appropriate refugia in transgenic cropping systems (see Sarfraz et al., 2006 for review).

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive cosmopolitan insect pests of brassicaceous crops. Although over 135 parasitoid species attack various life stages of *P. xylostella*, most control worldwide is achieved by relatively few hymenopteran species belonging to the ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus*. The larval parasitoids *Diadegma insulare* (Cresson) and *Microplitis plutellae* (Muesebeck) dominate in North America, and can often effectively suppress *P. xylostella* infestations (see Sarfraz et al., 2005 for review).

Understanding within-field temporal and spatial distributions of *P. xylostella* parasitoids in relation to distributions of their hosts could lead to ways of enhancing their effectiveness as biological control agents in an IPM program. The spatial and temporal distributions of various insect pests in canola have been investigated in the past decade, but studies focusing on spatio-temporal dynamics of *P. xylostella* and its parasitoid fauna have rarely been undertaken. Ulmer et al. (2004) described general attributes of the spatio-temporal distribution patterns of *P. xylostella* and *D. insulare* in canola, but did not investigate underlying causal mechanisms for observed distributions. Plant nutritional quality can influence some developmental and performance parameters of *P. xylostella* and *D. insulare* (Fox et al. 1990; Sarfraz et al. 2007), but it is not known whether host plant quality is a principal mechanism governing field distributions. In this study, adult *P. xylostella*, *D. insulare*, and *M. plutellae* were collected weekly from spatially referenced sampling points within commercial fields of spring canola (*B. napus*). Corresponding spatially referenced plant tissue samples were also collected and analyzed for nutrient contents, to investigate relationships among field populations, associated parasitoids, and host plant nutritional quality. Insect counts were mapped using geographical information system (GIS) technology, and analyzed using the spatial analysis with distance indices (SADIE) software. The objective of my study was to understand underlying mechanisms for observed distributions of the herbivore and its parasitoids to improve forecasting of areas of high populations within commercial fields so that integrated management strategies could be optimized.

8.2 Materials and Methods

8.2.1 Study Design and Insect Sampling

Grids were established in 2005 in each of two commercial fields of *B. napus* located near Lethbridge, Alberta (112° 47'W; 49° 40'N). In Field 1 and Field 2, 88 and 80 sampling plots were established respectively, each measuring 10 x 10 m, and a yellow bowl trap sampler was placed in the centre of each plot. The bowl trap samplers (15-cm diameter, 9-cm depth) followed the design of Dosdall et al. (2006). In Field 1, the grid consisted of eight rows with 11 samplers per row whereas the grid in Field 2 consisted of eight rows with 10 samplers per row. Bowl traps were set in position on 16 June 2005 when the crops were in bud to early flowering (Growth Stage 55 of Thomas, 2002), and the traps were adjusted weekly on their anchor posts so they remained at the level of the crop canopy throughout the study. Each bowl trap was filled with a mixture of water and propylene glycol (1:1), and refilled as needed. Samples were collected at weekly intervals for six weeks until the crop matured. Bowl trap contents were strained through a fine mesh net, and the filtered insect specimens were preserved in 70% ethanol. Samples were stored in the laboratory, and adult *P. xylostella*, *D. insulare* and *M. plutellae* were counted and recorded.

In each grid, *P. xylostella* adults and larvae were also sampled on 13 July (late flowering; Growth Stage 67 of Thomas 2002) using insect sweep nets. Sampling involved taking 10, 180° sweep net samples from each grid plot in the vicinity of the bowl traps, placing each sample in a labeled plastic bag, and storing samples temporarily in 70% ethanol until specimens were identified, counted, and recorded (for detailed procedures see Dosdall et al., 2006).

8.2.2 Tissue Nutrient Analysis

On 13 July (late flowering; Growth Stage 67 of Thomas 2002), leaf samples were taken from plants following a W-pattern within each plot and brought to the laboratory. Leaf samples were air-dried at room temperature, ground and subjected to nutrient analysis. The combustion method (AOAC-990.03) was followed for determination of total nitrogen and sulfur (AOAC, 2003a) while calcium, phosphorous, potassium, magnesium and sodium were assessed by using the inductively coupled plasma spectroscopic method (AOAC-985.01) in Norwest Labs, Lethbridge, Canada (AOAC, 2003b).

8.2.3 Data Analyses

Numbers of *P. xylostella*, *D. insulare* and *M. plutellae* adults collected using bowl trap samples on each sampling date, and *P. xylostella* adults and larvae from sweep net collections were restructured to create separate ascii-grid matrices by date using statistical software (SAS Institute, 2004). The ascii files were then imported into ArcInfo GIS Software (Environmental System Research Institute, Redlands, CA, USA) to create GRIDS using the ASCIIGRID function. Grid size was set to 100 units, and the grid data were then converted to point functions using the GRIDPOINT function of ArcInfo. The point coverages were then interpolated back to GRIDS using the POINTINTERP command. Interpolation was done by resampling the grid size to 10 units and interpolating to a radius of 200 units using exponential distance weighted interpolation with a smoothing factor applied to the point values. The process examines point data in relation to values from its nearest neighbor to estimate how values decline with distance from the point in question. On each sampling date, the spatial distributions of *P. xylostella*, *D. insulare* and *M. plutellae* were converted to contour maps of density and interpolated using ArcInfo GIS software as described by Dosdall et al. (2006) for the cabbage seedpod weevil and its parasitoids. A similar procedure was followed for GIS analysis and spatial distribution of leaf tissue nutrient contents.

SADIE software (Spatial Analysis by Distance IndicEs) was used to analyze spatial distribution patterns (degree of clustering) of *P. xylostella*, *D. insulare* and *M. plutellae* adults for each bowl trap sampling date, distributions of *P. xylostella* larvae from sweep net sampling, and individual leaf tissue nutrients. SADIE performs permutations of observed insect counts among sampling units and assesses observed arrangements in species count data by tests of randomization. The technique identifies areas of a patch or gap; a patch is a region of relatively large insect counts close to each other, and a gap is a region of relatively small insect counts close to each other (Perry, 1998). An index is assigned at each location that quantifies the degree to which a sampled count at the location contributes toward clustering of the population. To quantify the degree of clustering, the distance to regularity (D) is calculated, which is the minimum total distance that individuals in an observed arrangement must be moved between sampling units to produce a uniform or regular spatial distribution. The higher the value of D, the more the observed arrangement of counts is spatially aggregated. The degree of non-randomness was quantified by comparing the observed D with the mean expected distance to regularity (E_a) of randomization results for rearrangements in which the sampled counts first were randomly redistributed among the sampling locations (Perry, 1995, 1998). My analyses used the maximum number of randomizations possible within the SADIE program (i.e., 5,967) (see Dosdall et al., 2006).

Spatial patterns of single sets of insect counts on a given date from different locations within the grid were determined from the SADIE statistics with the SADIESHELL version (Rothamsted Experimental Station, Harpenden, Hertfordshire, UK). To measure aggregation patterns over the entire sample area, the overall aggregation index, I_a, was calculated as $I_a = D/E_a$. A value of $I_a = 1.0$ indicates a spatially random pattern, $I_a > 1.0$ indicates a more aggregated pattern, and $I_a < 1.0$ suggests a more regular pattern. The associated probability (P_a) was calculated from the formal randomization tests (Perry, 1995, 1998). To distinguish among patterns, the subsidiary index, J_a, was calculated. A value of $J_a > 1.0$ indicates a single major cluster, but $J_a < 1.0$

indicates two or more clusters. I also computed δ, a means of quantifying the degree to which an observed set of counts of individuals in a population occupies the edge or the centre of the area defined by the sample units. It refers to the distance between the two centroids, *P* and *C*. The computation determines the distance from the centroid of the sample units. The spatial analysis computes the location *P* from the x and y co-ordinates of sample units as the 'middle' of the sample, and location *C*, as the centroid of the counts. The value of δ is the distance between *P* and *C* (Perry and Klukowski, 1997).

Chi-square (χ^2) tests were performed to determine the variations in frequency distributions of *P. xylostella*, *D. insulare* and *M. plutellae* over the entire sampling period. Variations between growth stages (sampling dates) were also investigated when overall frequency distributions varied significantly ($P < 0.05$). *T*-tests (PROC TTEST) were also performed for pair-wise comparisons between catches per trap of *D. insulare* and *M. plutellae* on each sampling date (SAS Institute, 2004). Spatial associations among distinct but related data sets of insect distributions within the grids were assessed within the SADIE system. The method described by Winder et al. (2001) and Perry and Dixon (2002) calculates similarities in clustering indices of two sets of data, in this case between the occurrences of *P. xylostella*, their parasitoids, and between leaf tissue nutrients. The method assigns individual sample units to specific aggregation measures, thereby quantifying the extent that a particular unit contributed toward aggregation. The associations were evaluated by correlating the specific aggregation measures and the results were expressed as Pearson Correlation Coefficient statistics as described by Dosdall et al. (2006) for the cabbage seedpod weevil and its parasitoids.

8.3 Results

8.3.1 Temporal and Spatial Distributions of *P. xylostella*

Temporal consistency of distributions of *P. xylostella* adults varied significantly during the entire crop season in Fields 1 and 2 ($\chi^2 = 39.589$; $P < 0.0001$ and $\chi^2 = 29.758$; $P < 0.0001$ respectively). All sequential comparisons of weekly frequency distributions were non-significant ($P > 0.05$) except for week 1 vs. week 2 in Field 2 ($\chi^2 = 5.973$; $P = 0.0145$). In both fields, catches per trap were greatest during early flowering but populations became relatively stable during the late flowering and pod enlargement stages (Table 8-I; Figure 8-I). In Field 1, significant spatial aggregations of *P. xylostella* adults were detected during early flowering when population densities were high, but uniform spatial patterns were observed during mid flower, late flower and pod enlargement when population densities were low. The values of the subsidiary index I_a greater than unity suggested that single major clusters frequently occurred in Field 1 (Table 8-I). In Field 2, spatial distributions of adults were more frequently uniform (16-23 June, 23-30 June, 1-7 July, and 21-27 July) than aggregated (7-14 July and 14-21 July). Multiple clusters were present during mid flower ($J_a = 1.00$; $I_a = 1.04$) whereas single major clusters occurred during the remainder of the growth stages (Table 8-I). *Plutella xylostella* larval counts per sweep net sample indicated that multiple clustering was present in both fields with a strong aggregation in Field 1 ($\delta = 0.33$; $I_a = 1.53$; $P_a = 0.009$) and a marginal aggregation in Field 2 ($\delta = 0.25$; $I_a = 1.29$; $P_a = 0.058$) (Table 8-II; Figure 8-II).

8.3.2 Temporal and Spatial Distributions of *D. insulare*

During the entire crop season, weekly frequency distributions of *D. insulare* adults did not vary significantly in Fields 1 and 2 ($\chi^2 = 6.37$; $P = 0.2719$ and $\chi^2 = 8.473$; $P = 0.1320$ respectively); however, populations were relatively more abundant in early flower than later in crop development (Table 8-III; Figure 8-III). In both fields, spatial distributions of wasps were more frequently uniform (23-30 June, 1-7 July, 7-14 July, 14-21 July and 21-27 July) than aggregated (16-23 June) (Table 8-III). Further, *D. insulare* spatial distributions were significantly associated with those of its host *P. xylostella* in Fields 1 and 2 ($r = 0.31$, $P = 0.02$ and $r = 0.26$, $P = 0.03$ respectively).

8.3.3 Temporal and Spatial Distributions of *M. plutellae*

Weekly frequency distributions of *M. plutellae* adults did not vary significantly during the entire crop growing season in Fields 1 and 2 ($\chi^2 = 0.430$; $P = 0.9799$ and $\chi^2 = 0.636$; $P = 0.8819$ respectively). In both grid sites, spatial distribution patterns of *M. plutellae* were mostly uniform, but aggregated patterns were detected during mid flower in Field 1 (Table 8-IV; Figure 8-IV). *Microplitis plutellae* exhibited spatial association with their host *P. xylostella* in Field 1 ($r = 0.23$, $P = 0.05$) but not in Field 2 ($r = -0.03$, $P = 0.82$).

8.3.4 Temporal Distributions: Comparison between *D. insulare* and *M. plutellae*

In Field 1, *D. insulare* counts per trap were significantly higher than *M. plutellae* on 16-23 June and 23-30 June. A non-significant difference was observed between *D.*

insulare and *M. plutellae* populations from mid flower through pod enlargement stages of canola (Table 8-V). In Field 2, catches per trap of *D. insulare* were significantly higher than *M. plutellae* during the early flower, mid flower, and pod enlargement stages, but non-significant differences occurred during late flowering (Table 8-V).

8.3.5 Spatial Distributions of Leaf Tissue Nutrients: Associations with Distributions of *P. xylostella*, *D. insulare* and *M. plutellae*

For Field 1, concentrations of nitrogen, phosphorous, potassium, sulfur and sodium in leaf tissue were significantly aggregated whereas contents of calcium and magnesium were uniformly distributed within the sample area. Nitrogen, phosphorous, potassium and sodium contents exhibited multiple clustering, but sulfur exhibited a single major cluster (Table 8-VI; Figure 8-V). For Field 2, most foliar nutrient contents were strongly aggregated, but phosphorous and sulfur concentrations were relatively uniformly distributed. Multiple clusters were observed for nitrogen, phosphorous, sulfur and sodium concentrations whereas the remainder of nutrients exhibited single major clusters in Field 2 (Table 8-VI).

In Field 1, significant spatial associations occurred between *P. xylostella* adults and sulfur, *P. xylostella* larvae and sulfur, *P. xylostella* larvae and phosphorous, *P. xylostella* adults and potassium, *D. insulare* and sulfur, *D. insulare* and potassium, and *M. plutellae* and potassium (Table 8-VII). In Field 2, spatial associations were significant between *P. xylostella* adults and nitrogen, *P. xylostella* adults and potassium, *P. xylostella* adults and magnesium, *P. xylostella* adults and sodium, *P. xylostella* larvae and sodium, *D. insulare* and phosphorous, *D. insulare* and sodium, and *M. plutellae* and sodium (Table -VII).

8.4 Discussion

Extensive sampling of *P. xylostella* from points arranged in grid patterns in portions of commercial canola fields, and the mapping and analysis of their spatial distributions over time, has generated a comprehensive picture of the pattern of crop colonization by this pest. The present study does not provide direct evidence pertaining to the movements of individual *P. xylostella*; however, it does reveal a complex pattern of crop colonization by this pest, with invasions on multiple fronts, significant aggregations on different scales, and a simultaneous decline of populations at most sampling points as the crop season progressed.

The extant distribution patterns of *P. xylostella* in commercial fields reflect the unique dispersal history of this species. Although some evidence exists that *P. xylostella* can overwinter in Alberta (Dosdall, 1994), most populations in western Canada originate from influxes from southerly regions (Smith and Sears, 1982), with adults borne northward on air flow trajectories sometimes extending thousands of kilometers (Dosdall et al. 2004). Typical western Canadian canola production systems consist of very large commercial fields often extending over hundreds of hectares. *Plutella xylostella* exhibit pre-alighting recognition behavior and orient toward their preferred host plants based on olfactory cues whilst in flight (Bukovinszky et al., 2005). Immigrating moths may land in various areas of the crop depending on wind speed and direction and plant quality, thereby leading to the patches of initial infestations that, according to the present study, will further spread into the crop later in the season. Individual *P. xylostella* adults can disperse, on average, only 13 to 35 m within a crop (Cameron et al., 2002; Jianhua et al., 2003), so the initial pattern of deposition has importance for determining areas of high density within crops, and can cause aggregated distributions. Aggregated *P. xylostella* distributions were also detected in an earlier study (Ulmer et al., 2004). Contrary to the present findings, however, *P. xylostella* distributions were reported to remain consistent, with areas of relatively high densities remaining high throughout the season (Ulmer et al., 2004). In the present study, visual inspections of the GIS maps together with values of δ indicate that *P. xylostella* exhibited some tendency of dominant edge infestations, although it varied somewhat between fields (Figure 8-I; Table 8-I). Similar to the present results, *C. obstrictus* exhibited edge infestations early in the season and then spread toward the crop interior later in the season (Murchie et al., 1999; Dosdall et al., 2006).

Diadegma insulare exhibited significantly aggregated distributions during early flowering, but populations became more uniformly distributed later in the season. Similar to the present findings, aggregated distribution patterns for *D. insulare* were observed in canola by Ulmer et al. (2004). These researchers also reported that *D. insulare* populations reached their highest levels at the end of the season. By contrast, in the present study, *D. insulare* populations were highest during early flowering in both fields and numbers declined as the crop progressed (Table 8-II). These discrepancies could be attributable to several factors, including population densities of their *P. xylostella* hosts, ambient environmental conditions that can affect insect development, and arrival times of *D. insulare* on air currents that can affect parasitoid-herbivore-host plant synchronies.

Unlike *D. insulare*, *M. plutellae* distributions were mostly uniform during the entire sampling period. *Microplitis plutellae* are known to overwinter as pupae in western Canada and can provide biological control of *P. xylostella* early in the season (Putnam, 1968). *Microplitis plutellae* may have had aggregated distribution patterns early in the season in the present study, but this was not observed because sampling started only during the bud to early flower stage. By contrast, *D. insulare* is considered not to overwinter in western Canada (Putnam, 1968, 1978), and wasps are carried from southern regions by winds in spring along with their host, *P. xylostella*. *Diadegma insulare* distribution patterns similar to *P. xylostella* in the present study support this claim.

Both *D. insulare* and *M. plutellae* parasitize all four larval instars of *P. xylostella*, and the number of generations per year of both parasitoids corresponds to the number of generations of *P. xylostella* as one host larva supports only one parasitoid larva (Harcourt, 1960; Putnam, 1968). In the present study, *D. insulare* populations were significantly higher than *M. plutellae* during bud to early flowering in Field 1 and for most of the season in Field 2 (Table 8-V). This may be associated with a longer lifespan and higher overall fecundity of *D. insulare* than *M. plutellae*. On average, adult *M. plutellae* live for 20 days and produce 316 eggs per female whereas adult *D. insulare* live for 26 days and produce 814 eggs per female (Bolter and Laing, 1983). High parasitism levels result in high population densities of new generation wasps and earlier field studies reported higher parasitism levels by *D. insulare* than by *M. plutellae*. For instance, *D. insulare* accounted for ca. 45% of the total parasitism in 1992 in Alberta, while *M. plutellae* accounted for only ca. 14% of the total parasitism. A similar situation was observed in Saskatchewan with 30% parasitism by *D. insulare* and <8% by *M. plutellae*

(Braun et al., 2004). Similarly, 64.9% of the total parasitism was caused by *D. insulare* and 33.0% by *M. plutellae* in Geneva, New York (Xu et al., 2001).

Both *D. insulare* and *M. plutellae* are attracted to brassicaceous plants damaged by host *P. xylostella* larvae (Mitchell et al., 1999; Xu et al., 2001) suggesting that a high density of parasitoids could be indicative of high densities of *P. xylostella* larvae. The close coincidence of host and parasitoid spatial distributions in the present study indicates that even a spatially targeted application of insecticide would likely kill the parasitoids of *P. xylostella* as well as the host species. However, highest densities of parasitoids were not always coincident with high densities of *P. xylostella*, and some areas of high pest populations were not exploited by parasitoids (Figures 8-I, 8-II, 8-III and 8-IV) suggesting that such areas could serve as refugia for the parasitoids if only high *P. xylostella* infestation areas are treated with insecticides. In addition, certain insecticides safe for parasitoid pupae (Haseeb and Amano, 2002; Xu et al., 2004; Sarfraz and Keddie, 2005) provide an opportunity to use such selective insecticides when the majority of parasitoids are in the pupal stage.

The strong effect of leaf sulfur and nitrogen on spatial distributions of *P. xylostella* in one of the two fields suggests that plant patches with high sulfur and/or nitrogen contents may serve as 'hot-spots' for herbivore infestations. Both sulfur and nitrogen are important components of defensive compounds (glucosinolates) in Brassicaceae, and some of these compounds act as oviposition and feeding stimulants for *P. xylostella* (Gupta and Thorsteinson, 1960; Reed et al., 1989; Marazzi et al., 2004). Earlier studies indicated that sulfur-deficient plants were less attractive for *P. xylostella* oviposition. For instance, oviposition was significantly higher on sulfur-fertilized

Brassica species than on sulfur-deficient controls (Gupta and Thorsteinson, 1960). Similarly, numbers of eggs laid on artificial leaves treated with the leaf-surface extracts of plants grown under normal sulfur (field concentration) were significantly higher than on artificial leaves sprayed with sulfur-free plant extracts (Marazzi et al., 2004). The present findings regarding the *P. xylostella* association with total leaf nitrogen are also partially in accordance with the previous reports. For example, *P. xylostella* abundance was marginally correlated with total leaf nitrogen in a small-scale plot experiment (Fox et al., 1990).

The discovery of a relationship between sulfur and *P. xylostella* in Field 1, but not in Field 2, and a relationship between nitrogen and *P. xylostella* in Field 2 but not in Field 1, may be related to differences in plant tissue contents of the two nutrients in each field. In Field 1, concentrations of sulfur in leaf tissue varied considerably across the grid, from less than 1.6% to 2.8%, but in Field 2 these levels were very uniform and mostly less than 1.6%, with only small patches reaching 2.2% (Figure 8-V). In Field 2, a strong gradient was observed in nitrogen contents across the grid so that concentrations varied from less than 3.6% to 6.0%, but in Field 1, nearly the entire grid had concentrations of less than 3.6% (Figure 8-V). Although the present results are inconclusive, they point to questions that should be investigated further in replicated field studies where different soil fertility levels can be manipulated. Further, in commercial field situations, there are many interacting factors that could not be measured in the present study. For example, weed infestation data were not recorded, yet it is known that weeds can interact with insect behavior (Horn, 1981; Shellhorn and Sork, 1997; Finch and Collier, 2000; Norris and Kogan, 2000; Dosdall et al., 2003; Oyediran et al., 2007). Similarly, there may have been differences in plant density between different grid plots, and these could also interact with nutrients and subsequently with herbivore abundance. Nevertheless, the linkage between sulfur and nitrogen and *P. xylostella* has been carefully examined in laboratory situations (Gupta and Thorsteinson, 1960; Marazzi et al., 2004; Sarfraz et al., 2007) and concentrations of these nutrients appear to govern field distributions to some extent.

The spatial and temporal distribution patterns of *P. xylostella* and associated parasitoids in canola crops have implications for the design of accurate sampling procedures to support decisions on the need for pest control measures. In general, the assessment of *P. xylostella* infestations is made by trapping adults or counting numbers of larvae on randomly selected plants (Ulmer et al., 2004). Where populations are aggregated, traditional sampling may not provide accurate estimates of the pest and parasitoid populations, and the present study suggests that population assessment methods should be based on more rigorous research focusing on the spatial distribution of *P. xylostella*. A similar strategy was proposed for sampling *C. obstrictus* in winter canola (Ferguson et al., 2000). The aggregated distributions of *P. xylostella* adults also suggest a potential for spatial targeting of insecticide treatments to areas where pests are more abundant as proposed for *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Weisz et al., 1995) and *C. obstrictus* (Ferguson et al., 2000), thereby maximizing control of the pest while minimizing their adverse effects. For instance, spatial aggregations of *P. xylostella* during bud to early flower, and the inability of parasitoids to exploit all high pest density areas within a crop suggest that, if necessary, high infestation areas could be targeted for selective insecticide treatments.

Table 8-I. Characterization of spatial and temporal distributions of adults of *Plutella xylostella* from 88 (Field 1) and 80 bowl traps (Field 2) sampled weekly in 2005 in grid patterns within commercial fields of *Brassica napus*. Spatial distribution indices, calculated with the SADIE procedure, are presented for *P. xylostella* adults.

Field	Sample period	Canola growth stage	Mean adults per trap (± S.E.)	δ[1] (m)	I_a[2]	P_a[3]	J_a[4]
1	16 – 23 June	Early Flower	15.79 (1.88)	1.05	1.49	0.013	1.02
1	23 – 30 June	Early Flower	10.89 (1.13)	0.87	1.44	0.022	1.04
1	1 – 7 July	Mid Flower	3.49 (0.21)	0.18	0.91	0.658	1.02
1	7 – 14 July	Late Flower	0.91 (0.13)	0.43	0.88	0.778	1.11
1	14 – 21 July	Pod Enlargement	0.67 (0.10)	0.56	0.88	0.774	1.15
1	21 – 27 July	Pod Enlargement	0.22 (0.05)	0.59	0.93	0.612	1.23
2	16 – 23 June	Early Flower	12.79 (1.55)	0.53	1.07	0.288	1.01
2	23 – 30 June	Early Flower	3.06 (0.31)	0.40	0.98	0.478	1.04
2	1 – 7 July	Mid Flower	6.18 (0.52)	0.25	1.04	0.328	1.00
2	7 – 14 July	Late Flower	1.20 (0.14)	0.64	1.40	0.023	1.16
2	14 – 21 July	Pod Enlargement	0.40 (0.07)	1.31	1.42	0.013	1.17
2	21 – 27 July	Pod Enlargement	0.21 (0.05)	0.67	0.94	0.585	1.16

[1] The distance between the two centroids, *P* and *C* (see text for details)

[2] Values of I_a indicate spatial patterns; $I_a = 1$ indicates a random distribution, $I_a > 1$ indicates aggregation, and $I_a < 1$ indicates a uniform pattern within the sample area

[3] Associated *P* values; aggregations are significant at $P < 0.05$

[4] Values of $J_a \leq 1$ indicate the presence of multiple clusters when $I_a > 1$

Table 8-II. Mean (± S.E.) adults and larvae of *Plutella xylostella* per 10 sweep net samples from 88 (Field 1) and 80 sites (Field 2) sampled on 13 July 2005 in grid patterns within commercial fields of *Brassica napus*. Spatial distribution indices, calculated with the SADIE procedure, are presented for *P. xylostella* adults and larvae.

Field	Life stage	Mean per 10 sweeps (± S.E.)	δ^1 (m)	I_a^2	P_a^3	J_a^4
1	Adults	0.86 (0.12)	0.51	0.99	0.444	1.10
1	Larvae	175.80 (7.33)	0.33	1.53	0.009	1.00
1	Total	176.66 (7.72)	0.36	1.56	0.009	1.08
2	Adults	0.70 (0.11)	1.15	1.43	0.021	1.03
2	Larvae	88.20 (5.14)	0.25	1.29	0.058	1.00
2	Total	88.90 (5.16)	0.24	1.28	0.063	1.00

[1] The distance between the two centroids, *P* and *C* (see text for details)

[2] Values of I_a indicate spatial patterns; $I_a = 1$ indicates a random distribution, $I_a > 1$ indicates aggregation, and $I_a < 1$ indicates a uniform pattern within the sample area

[3] Associated *P* values; aggregations are significant at $P < 0.05$

[4] Values of $J_a \leq 1$ indicate the presence of multiple clusters when $I_a > 1$

Table 8-III. Characterization of spatial and temporal distributions of adults of *Diadegma insulare* from 88 (Field 1) and 80 bowl traps (Field 2) sampled weekly in 2005 in grid patterns within commercial fields of *Brassica napus*. Spatial distribution indices, calculated with the SADIE procedure, are presented for *D. insulare* adults.

Field	**Sample period**	**Canola growth stage**	**Mean *D. insulare* adults per trap (± S.E.)**	**δ[1] (m)**	**I_a[2]**	**P_a[3]**	**J_a[4]**
1	16 – 23 June	Early Flower	1.99 (0.23)	1.25	1.78	0.002	1.06
1	23 – 30 June	Early Flower	0.45 (0.10)	0.91	1.06	0.282	1.19
1	1 – 7 July	Mid Flower	0.17 (0.04)	0.86	1.10	0.223	1.22
1	7 – 14 July	Late Flower	0.06 (0.02)	1.75	0.98	0.455	1.13
1	14 – 21 July	Pod Enlargement	0.06 (0.02)	1.80	0.99	0.451	1.36
1	21 – 27 July	Pod Enlargement	0.03 (0.03)	2.12	0.90	0.673	1.16
2	16 – 23 June	Early Flower	2.95 (0.57)	1.73	1.76	0.001	1.19
2	23 – 30 June	Early Flower	0.99 (0.15)	0.09	0.90	0.679	1.16
2	1 – 7 July	Mid Flower	0.34 (0.07)	0.95	1.02	0.376	1.12
2	7 – 14 July	Late Flower	0.10 (0.03)	0.67	0.84	0.856	1.05
2	14 – 21 July	Pod Enlargement	0.14 (0.04)	1.16	1.03	0.366	1.26
2	21 – 27 July	Pod Enlargement	0.08 (0.03)	0.60	0.75	0.989	1.35

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[1] The distance between the two centroids, *P* and *C* (see text for details)

[2] Values of I_a indicate spatial patterns; $I_a = 1$ indicates a random distribution, $I_a > 1$ indicates aggregation, and $I_a < 1$ indicates a uniform pattern within the sample area

[3] Associated *P* values; aggregations are significant at $P < 0.05$

[4] Values of $J_a \leq 1$ indicate the presence of multiple clusters when $I_a > 1$

Table 8-IV. Characterization of spatial and temporal distributions of adults of *Microplitis plutellae* from 88 (Field 1) and 80 bowl traps (Field 2) sampled weekly in 2005 in grid patterns within commercial fields of *Brassica napus*. Spatial distribution indices, calculated with the SADIE procedure, are presented for *M. plutellae* adults.

Field	Sample period	Canola growth stage	Mean *M. plutellae* adults per trap (± S.E.)	δ[1] (m)	I_a[2]	P_a[3]	J_a[4]
1	16 – 23 June	Early Flower	0.23 (0.05)	1.00	1.01	0.389	1.19
1	23 – 30 June	Early Flower	0.09 (0.03)	0.74	0.86	0.809	1.15
1	1 – 7 July	Mid Flower	0.11 (0.04)	2.78	1.44	0.022	1.11
1	7 – 14 July	Late Flower	0.06 (0.02)	2.62	1.25	0.085	1.21
1	14 – 21 July	Pod Enlargement	0.01 (0.01)	3.50	0.98	0.560	—
1	21 – 27 July	Pod Enlargement	0.00 (0.00)	—[5]	—	—	—
2	16 – 23 June	Early Flower	0.34 (0.08)	0.99	1.04	0.348	1.04
2	23 – 30 June	Early Flower	0.10 (0.04)	1.35	0.93	0.609	1.36
2	1 – 7 July	Mid Flower	0.10 (0.04)	1.70	1.19	0.127	1.46
2	7 – 14 July	Late Flower	0.14 (0.04)	0.58	0.88	0.758	1.43
2	14 – 21 July	Pod Enlargement	0.00 (0.00)	—	—	—	—
2	21 – 27 July	Pod Enlargement	0.00 (0.00)	—	—	—	—

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[1] The distance between the two centroids, *P* and *C* (see text for details)

[2] Values of I_a indicate spatial patterns; $I_a = 1$ indicates a random distribution, $I_a > 1$ indicates aggregation, and $I_a < 1$ indicates a uniform pattern within the sample area

[3] Associated *P* values; aggregations are significant at $P < 0.05$

[4] Values of $J_a \leq 1$ indicate the presence of multiple clusters when $I_a > 1$

[5] No insects were found at any sample point

Table 8-V. Pair-wise comparison (*t*-test) between adults of *Diadegma insulare* and *Microplitis plutellae* from 88 (Field 1) and 80 bowl traps (Field 2) sampled weekly in 2005 in grid patterns within commercial fields of *Brassica napus*.

Field	Sample period	Canola growth stage	*t*	*P*
1	16 – 23 June	Early Flower	8.15	<0.0001
1	23 – 30 June	Early Flower	3.88	0.0001
1	1 – 7 July	Mid Flower	1.20	0.2306
1	7 – 14 July	Late Flower	0.00	1.0000
1	14 – 21 July	Pod Enlargement	1.67	0.0977
1	21 – 27 July	Pod Enlargement	1.38	0.1703
2	16 – 23 June	Early Flower	6.18	<0.0001
2	23 – 30 June	Early Flower	6.32	<0.0001
2	1 – 7 July	Mid Flower	3.01	0.0031
2	7 – 14 July	Late Flower	-0.64	0.5254
2	14 – 21 July	Pod Enlargement	3.29	0.0012
2	21 – 27 July	Pod Enlargement	2.53	0.0124

Table 8-VI. Concentrations (%) of various nutrients from 88 (Field 1) and 80 samples (Field 2) collected during late flowering in 2005 in grid patterns within commercial fields of *Brassica napus*. Spatial distribution indices, calculated with the SADIE procedure, are presented.

Field	Nutrient type	Nutrient Concentration (± S.E.)	δ[1] (m)	I_a[2]	P_a[3]	J_a[4]
1	Nitrogen	3.22 (0.06)	0.25	2.22	0.009	1.01
1	Phosphorous	0.38 (0.03)	0.33	2.41	<0.001	1.01
1	Potassium	3.01 (0.07)	0.30	2.46	<0.001	1.02
1	Sulfur	2.17 (0.04)	0.16	1.77	0.001	1.17
1	Calcium	5.66 (0.07)	0.08	1.25	0.086	1.19
1	Magnesium	0.77 (0.01)	0.07	1.16	0.166	1.14
1	Sodium	1.39 (0.01)	1.23	2.25	<0.001	1.02
2	Nitrogen	4.49 (0.08)	0.16	1.80	<0.001	1.01
2	Phosphorous	0.42 (0.01)	0.21	1.28	0.080	1.01
2	Potassium	3.19 (0.10)	0.32	2.15	<0.001	1.20
2	Sulfur	1.77 (0.03)	0.06	1.18	0.143	1.00
2	Calcium	4.06 (0.12)	0.32	1.94	<0.001	1.29
2	Magnesium	0.91 (0.03)	0.35	1.84	<0.001	1.23
2	Sodium	0.66 (0.03)	0.41	1.73	<0.001	1.03

[1] The distance between the two centroids, *P* and *C* (see text for details)
[2] Values of $I_a > 1$ indicate an aggregated spatial pattern within the sample area
[3] Associated *P* values; aggregations are significant at $P < 0.05$
[4] Values of $J_a \leq 1$ indicate the presence of multiple clusters when $I_a > 1$

Table 8-VII. Spatial associations among various nutrients and distributions of *Plutella xylostella* adults and larvae, *Diadegma insulare* and *Microplitis plutellae* from 88 (Field 1) and 80 samples (Field 2) collected in 2005 in grid patterns within commercial fields of *Brassica napus*. Pearson correlation coefficients (*r*) with associated probability (*P*) in parenthesis; significant associations are in bold.

Field	**Nutrient type**	***P. xylostella* adults**	***P. xylostella* larvae**	***D. insulare* adults**	***M. plutellae* adults**
1	Nitrogen	-0.06 (0.61)	-0.10 (0.44)	**-0.28 (0.02)**	-0.18 (0.14)
1	Phosphorous	-0.10 (0.39)	**0.29 (0.01)**	-0.10 (0.37)	-0.05 (0.65)
1	Potassium	**0.26 (0.02)**	0.14 (0.21)	**0.21 (0.04)**	**0.22 (0.04)**
1	Sulfur	**0.31 (0.02)**	**0.27 (0.03)**	**0.26 (0.04)**	0.15 (0.19)
1	Calcium	0.06 (0.61)	-0.03 (0.79)	0.04 (0.77)	0.06 (0.64)
1	Magnesium	0.20 (0.10)	-0.11 (0.41)	0.17 (0.22)	0.12 (0.35)
1	Sodium	-0.04 (0.71)	-0.12 (0.30)	-0.04 (0.74)	-0.10 (0.41)
2	Nitrogen	**0.27 (0.03)**	-0.06 (0.65)	-0.06 (0.65)	0.17 (0.19)
2	Phosphorous	0.09 (0.44)	-0.19 (0.11)	**0.31 (0.01)**	0.05 (0.71)
2	Potassium	**0.16 (0.02)**	0.02 (0.87)	0.01 (0.95)	-0.02 (0.90)
2	Sulfur	0.02 (0.87)	-0.20 (0.08)	0.08 (0.49)	-0.11 (0.36)
2	Calcium	0.13 (0.23)	-0.15 (0.18)	0.02 (0.87)	-0.04 (0.73)
2	Magnesium	**0.18 (0.05)**	-0.12 (0.28)	0.13 (0.27)	0.15 (0.20)
2	Sodium	**0.24 (0.03)**	**0.29 (0.02)**	**0.26 (0.03)**	**0.25 (0.04)**

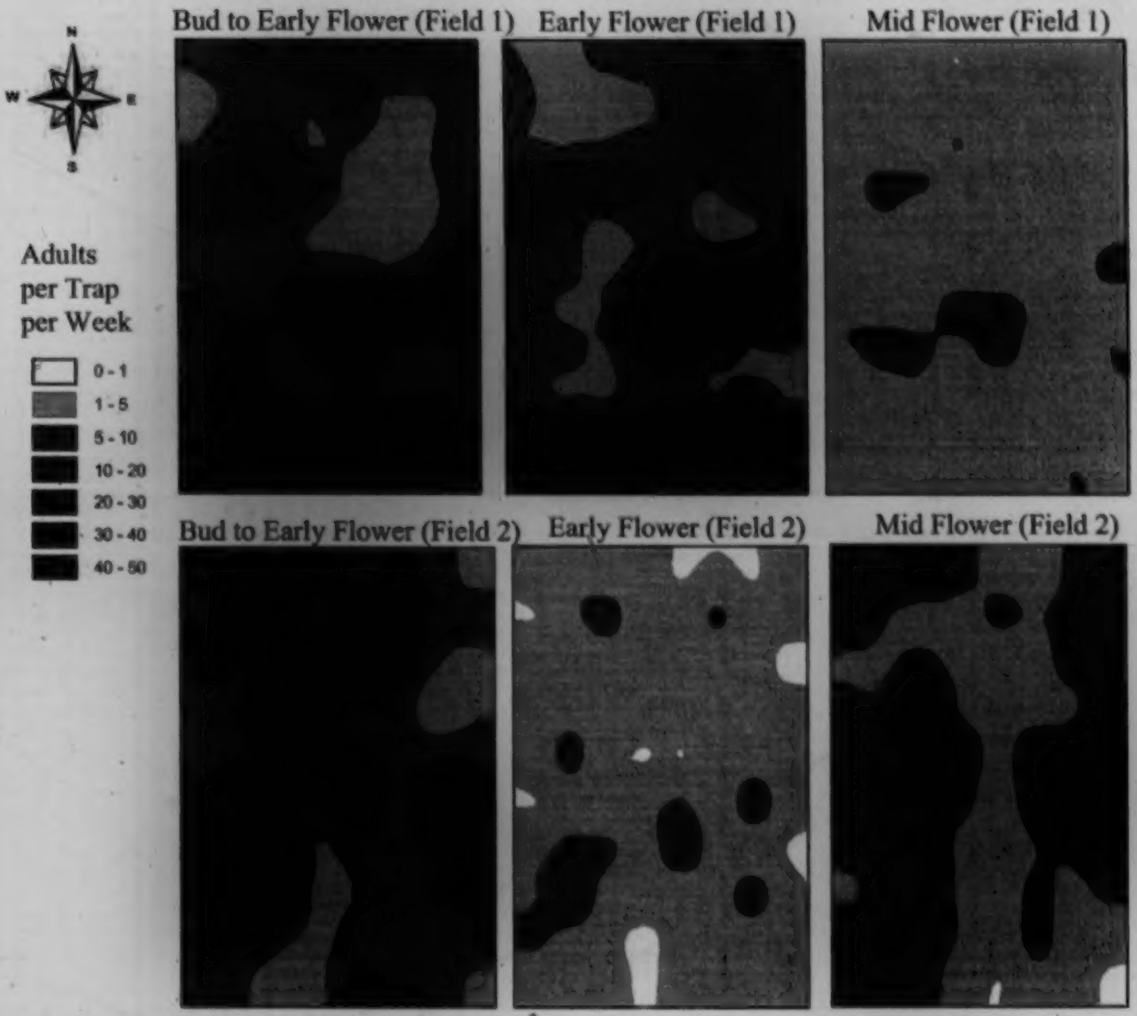


Figure 8-I. Distributions of *Plutella xylostella* adults interpolated from samples collected from 88 (Field 1) and 80 bowl traps (Field 2) in 2005 within grid areas of commercial crops of *Brassica napus* near Lethbridge, Alberta. The legend gives *P. xylostella* adults per bowl trap sample.

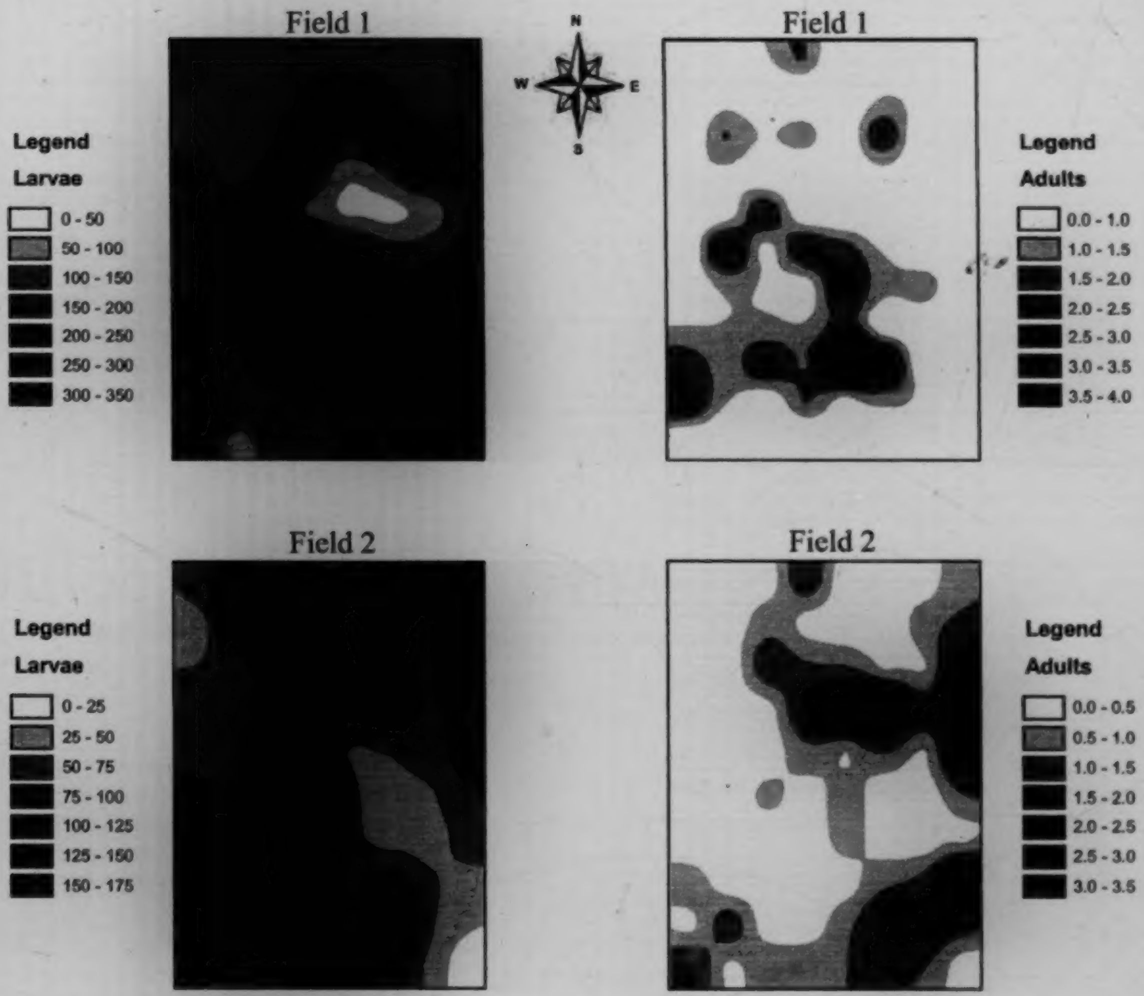
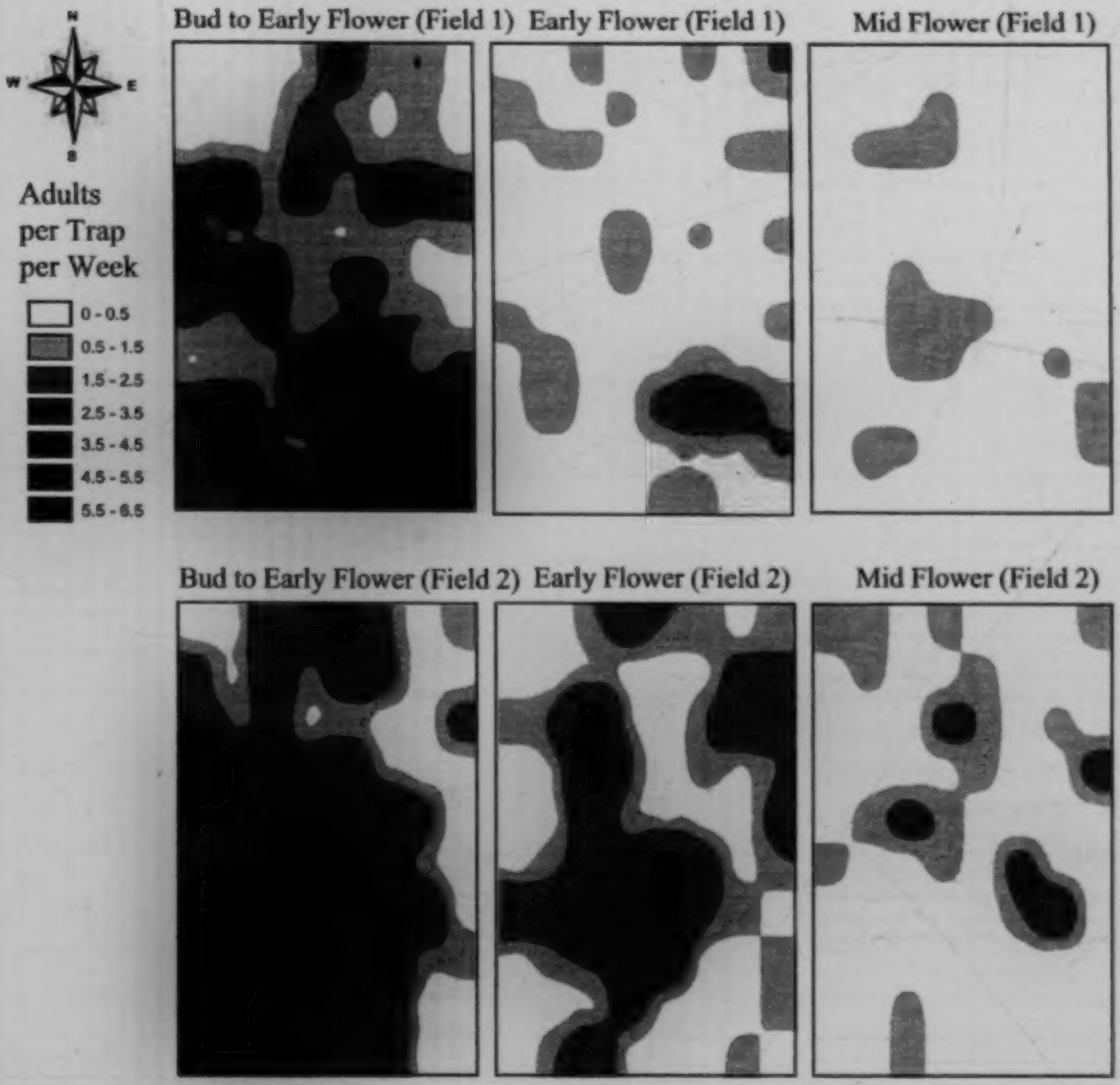


Figure 8-II. Distributions of *Plutella xylostella* larvae and adults interpolated from sweep net samples collected from 88 (Field 1) and 80 plots (Field 2) during late flowering in 2005 within grid areas of commercial crops of *Brassica napus* near Lethbridge, Alberta. The legends give *P. xylostella* larvae and adults per sweep net sample.



Bud to Early Flower (Field 1) Early Flower (Field 1) Mid Flower (Field 1)

Bud to Early Flower (Field 2) Early Flower (Field 2) Mid Flower (Field 2)

Figure 8-III. Distributions of *Diadegma insulare* adults interpolated from samples collected from 88 (Field 1) and 80 bowl traps (Field 2) in 2005 within grid areas of commercial crops of *Brassica napus* near Lethbridge, Alberta. The legend gives *D. insulare* adults per bowl trap sample.

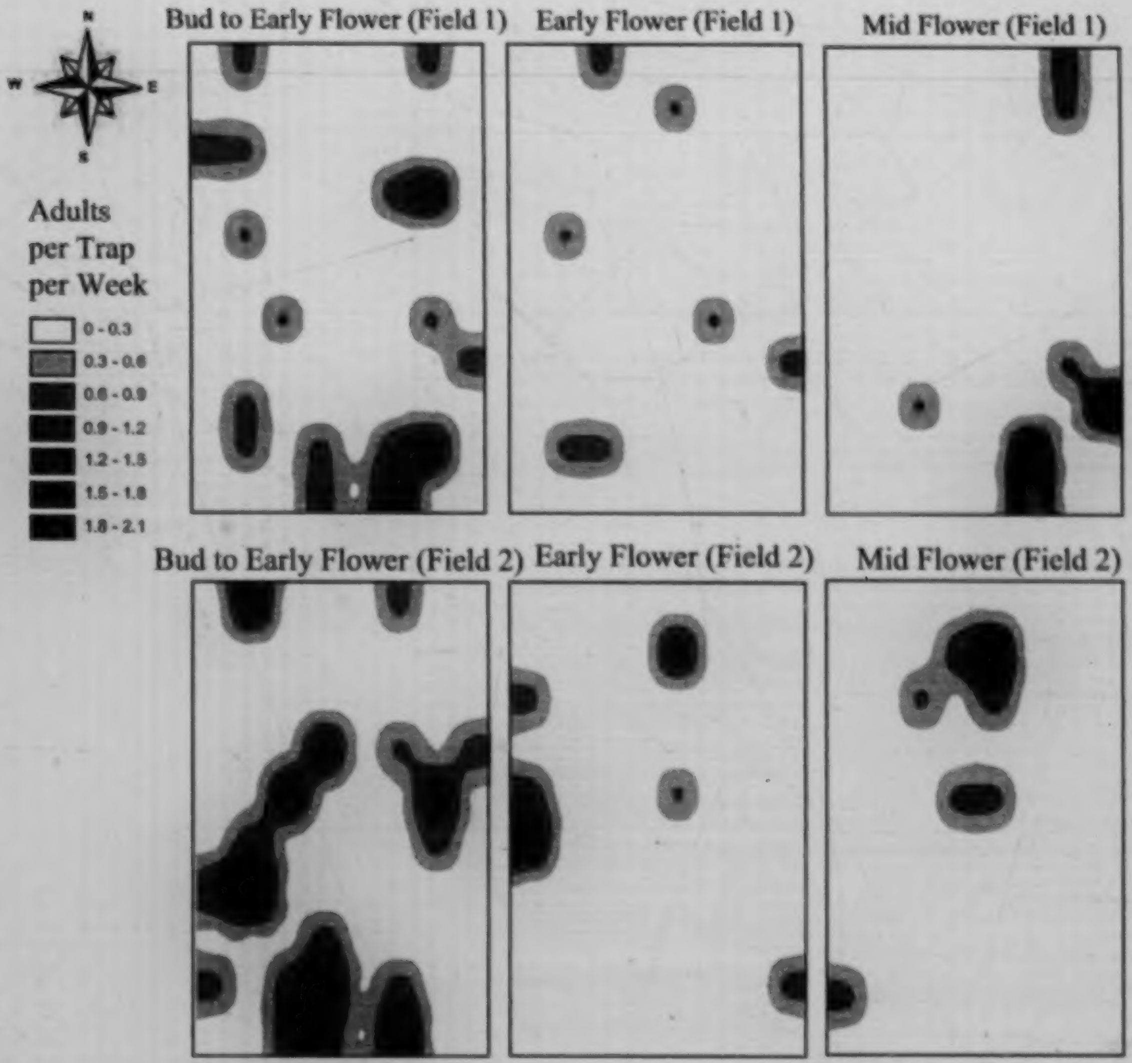


Figure 8-IV. Distributions of *Microplitis plutellae* adults interpolated from samples collected from 88 (Field 1) and 80 bowl traps (Field 2) in 2005 within grid areas of commercial crops of *Brassica napus* near Lethbridge, Alberta. The legend gives *M. plutellae* adults per bowl trap sample.

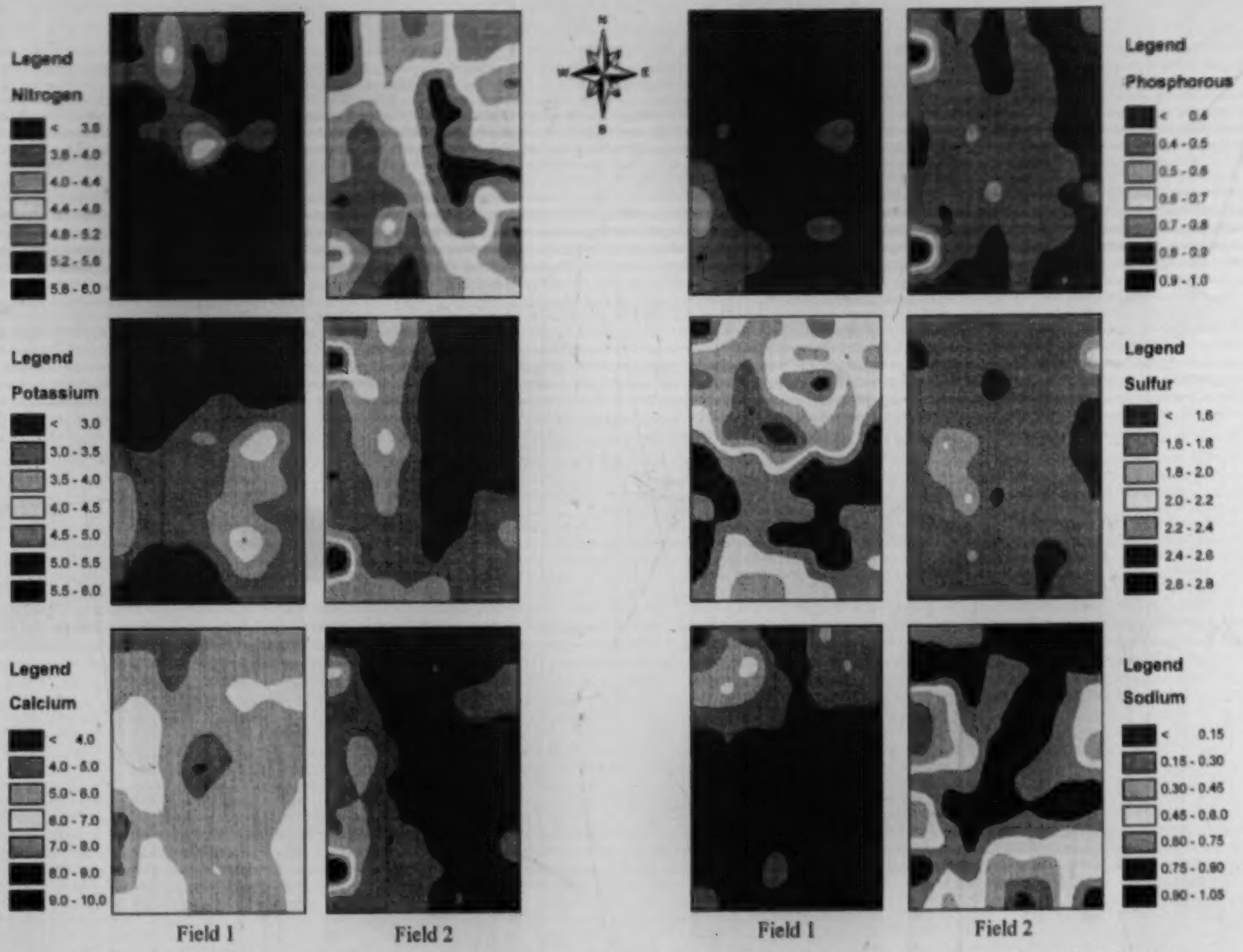


Figure 8-V. Spatial distributions of various nutrients interpolated from 88 (Field 1) and 80 samples (Field 2) collected during late flowering in 2005 within grid areas of commercial crops of *Brassica napus* near Lethbridge, Alberta. The legends give percent nutrient concentrations per leaf tissue sample.

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Chapter 9:

Concluding Discussion

9.1 Discussion

Different fertilizer applications significantly affected the nutrient contents of *B. napus* foliage, and this in turn affected preference and performance of *P. xylostella*. Females of diamondback moth discriminated among host plants subjected to different levels of soil fertility for oviposition, and tended to select plants on which pre-imaginal survival and development of their offspring was maximal, and on which new generation adults had highest longevity when their food was limited (Chapter 2). My findings support neither the plant stress nor the plant vigor hypothesis. Unfertilized plants of *B. napus* had low levels of nitrogen in their leaves and larvae of *P. xylostella* consumed more foliage, presumably to compensate for this nutrient deficiency. Further, unfertilized plants received fewer eggs and yielded lighter pupae compared with intermediate fertility regimes. Although plants grown at the highest fertilizer treatment had the greatest leaf nitrogen contents, such plants also received fewer eggs and reduced larval survival and herbivory occurred on these plants. The plant stress and the plant vigor hypotheses are likely two ends of a continuum of responses between insects and their host plants. Although some insect species appear to respond more favorably to stressed host plants (Mattson, 1980; White, 1984; Jones and Coleman, 1988), and others to vigorous plants (Fox et al., 1990; Price, 1991; Meyer and Root, 1996; Inbar et al., 2001; Chen et al., 2004; Dosdall et al., 2004; Heisswolf et al., 2005), *P. xylostella* responds better to plants grown at intermediate levels of fertilizer. My investigations indicated a complex set of interactions involving both bottom-up and top-down effects, which interact to affect host plant morphology and tissue nutrients, oviposition site selection by female herbivores, and fitness of new generation offspring (Chapter 2).

Investigation of tritrophic interactions involving *B. napus* subjected to varying levels of soil fertility, being fed upon by *P. xylostella* larvae under attack by *D. insulare* parasitoids, uncovered a complex series of bottom-up effects influencing the third trophic level (Chapter 5). Several parameters of the parasitoid indicated maximal fitness when *D. insulare* were reared on *P. xylostella* larvae that fed on plants fertilized at relatively high rates. In view of the extensive worldwide economic importance of this herbivore, and the vast areas of the planet seeded annually to brassicaceous crops, the question arises of whether fertility levels can be manipulated for enhanced biological control of *P. xylostella* with *D. insulare*. Canola stressed by low nutrient levels has poor seed yields, even in the absence of insect herbivory (Thomas, 1990), and although higher levels of soil fertility can predispose plants to increased infestations of *P. xylostella*, increased fertility also enhances development and survival of *D. insulare*. Vigorous, well nourished canola plants can better compensate for insect attack than plants under nutrient stress (Dosdall et al., 2004), so maintaining relatively high levels of soil fertility seems appropriate for integrated management of *P. xylostella* in canola. It is probable that this recommendation could also apply to other brassicaceous crops under attack by *P. xylostella*.

Comparisons of conventional (*B. napus* cv. Q2) and herbicide-tolerant (*B. napus* cvs. Liberty and Conquest) canola cultivars determined that females of *P. xylostella* deposited significantly more eggs on Liberty than on Conquest or Q2 (Chapter 3).

Although the present findings appear to indicate that *B. napus* cv. Liberty modified to express the *bar* gene also conferred greater susceptibility to ovipositing females, this cannot be concluded from this study. To answer this question would require comparisons of the same *B. napus* germplasm with and without the *bar* gene. However, eight Brassicaceae evaluated in my studies varied considerably in their suitabilities as hosts for *P. xylostella*, and these differences could perhaps be exploited by producers. For example, *B. oleracea* cannot compensate for larval feeding to the level of the other species I evaluated, so it may be appropriate to plant an alternative crop in areas where *P. xylostella* populations annually reach high densities. None of the Brassicaceae evaluated is a suitable candidate for *P. xylostella* trap cropping. However, *S. alba* plants were highly preferred by ovipositing females, consequently producers of this crop must be especially vigilant in monitoring *P. xylostella* infestations and applying chemical control measures when populations reach economic threshold densities. Finally, the greater propensity for *P. xylostella* females to oviposit on *S. alba* points to an opportunity to genetically modify this crop by introducing genes that express an antibiotic effect. The susceptible host could then become a dead-end trap for *P. xylostella*, and so enhance sustainable management of this pest.

Combined results reported in Chapters 3 and 6 have facilitated an improved understanding of both top-down effects of *P. xylostella* herbivory on different host plant species and cultivars, and bottom-up effects of the host plants on higher trophic levels. Some host plants respond to herbivory by compensatory development of greater quantities of root tissue (e.g., *B. napus*, *B. rapa*, *S. alba*), but others lack this capability (e.g., *B. oleracea*, *B. juncea*). Pre-imaginal developmental times of *P. xylostella*, pupal and adult weights, forewing areas, and adult longevities in the absence of food vary with host plant genotype, and the plant genotype on which herbivore hosts were reared is also linked with several fitness correlates of the *D. insulare*. Such thorough analysis of tritrophic relationships is rarely incorporated in the integrated management of pests, yet this study has shown that without understanding the complexities and interactions that occur in these systems, important management aspects can be missed. For example, survival of *P. xylostella* larvae and pupae is high when reared on *B. napus* cv. Q2, new generation adult body weights are relatively high, and plants of Q2 can respond to this herbivory by compensatory increases in root mass; however, parasitoids that develop on *P. xylostella* larvae reared on this host have low survival and new generation adults of *D. insulare* have poor longevities when held without food. To optimize integrated crop management, canola growers should then consider an alternate variety like *B. napus* cv. Conquest that also responds to herbivory by increasing its root mass, but leads to better survival of *D. insulare*, higher body weights of new generation parasitoids and greater longevity without food. Such interactions among crop genotype compensatory ability, and the performances of *P. xylostella* herbivores and their parasitoids likely exist in additional *Brassica* cropping systems besides canola. More extensive research into these relationships holds promise for enhancing our understanding of how to best to manage these systems.

The present findings have important implications regarding adaptive behavior of a crucifer specialist and ecological interactions among the plant species, the herbivore, and the parasitoid (Chapters 4 and 7). The physiological trade-off hypothesis suggests that host specialization is associated with a reduced ability to exploit the diversity of food plants in a given habitat (Fry, 1990; Bernays and Chapman, 1994). A variety of factors such as relative resource availability, plant biochemistry, insect mobility, population size and predation pressure may play important roles in the evolution of host specialization and subsequent maintenance of narrower diets. For instance, plant biochemistry provides constraints on specialists, in that they are often specifically stimulated by host compounds and are strongly deterred by non-host compounds (Bernays and Chapman, 1994). Like other specialist herbivores, oligophagy serves *P. xylostella* well when brassicaceous plants are abundant in a habitat. However, in times of biotic and/or abiotic stress, it has the capability to survive on suitable non-Brassicaceae. Evidently a component of the survival strategy of this species is that some segment of a given population seems to have fewer constraints on it (population heterogeneity), where certain females can oviposit on less preferred but acceptable food plants where their offspring can survive and reproduce. Insect population levels can further facilitate exploitation of novel host plants as there is some evidence to suggest that moths from high-density populations are more generalized in their host selection than moths from low-density populations (Bigger and Fox, 1997). Herbivores raised on unfavorable plants would be more adapted for emigration (Dent, 2000), and my research suggests that *P. xylostella* reared on suboptimal food plants develop characteristics such as a latent oviposition period and smaller body mass that may facilitate successful emigration.

Host expansion by the specialist herbivore, *P. xylostella*, to include the non-brassicaceous glucosinolate-containing species *C. hassleriana* and *T. majus* could be advantageous ecologically by enabling the herbivore to feed and persist when its more preferred hosts are unavailable. It could enable *P. xylostella* to expand its range to include habitats where *C. hassleriana* and *T. majus* occur but not Brassicaceae. The glucosinolate compounds that predominate in *C. hassleriana* and *T. majus* (glucocapparin and glucotropaeolin) are presumably detoxified by a similar biochemical pathway that enables the herbivore to detoxify the various long-chain glucosinolates that characterize Brassicaceae (Ratzka et al., 2002). Investigation of the fitness correlates of *D. insulare* developing on *P. xylostella* hosts reared on the different plant species uncovered evidence to indicate that some selection pressure could favor host range expansion by *P. xylostella* to include *C. hassleriana* or *T. majus* under field conditions. For selection to favor this host expansion, fitness of the parasitoid could be expected to diminish when its hosts were reared on non-brassicaceous versus brassicaceous hosts. I found that mean developmental times of *D. insulare* from egg to pupa and from egg to adult were significantly faster on *P. xylostella* hosts reared on *B. napus* than on *C. hassleriana* and *T. majus*, and adult body weights were lightest on hosts reared on *C. hassleriana*. On the other hand, survival of the parasitoid was unaffected by host plant species on which *P. xylostella* hosts were reared, and pupal weights were similar on *B. napus* relative to those on *C. hassleriana* and *T. majus*. Consequently, it is doubtful that less rapid pre-imaginal development on *C. hassleriana* and *T. majus* and smaller adult biomass of the parasitoid on *C. hassleriana* could provide sufficient advantage to *P. xylostella* to drive expansion of its host range to include one or both of these non-brassicaceous species. Moreover, *D. insulare* has a remarkable ability to adapt to host availability. *Diadegma insulare* is native to Neotropical regions (see Azidah et al., 2000; Sarfraz et al., 2005), but *P. xylostella* is believed native to Africa (Kfir, 1998), yet this parasitoid has become the most significant biological control agent of *P. xylostella* throughout North America

(Chapter 1) where it specializes on an introduced host species. With such a versatile biocontrol agent, it is probable that its effectiveness in exploiting *P. xylostella* hosts would be as successful on non-brassicaceous as on brassicaceous species.

Extensive sampling of *P. xylostella*, *D. insulare* and *M. plutellae* from points arranged in grid patterns in portions of commercial canola fields, and the mapping and analysis of their spatial distributions over time, has generated a comprehensive picture of the pattern of crop colonization by the pest and parasitoids. The present study does not provide direct evidence pertaining to the movements of individual insects; however, it does reveal a complex pattern of crop colonization by them, with invasions on multiple fronts, significant aggregations on different scales, and a simultaneous decline of populations at most sampling points as the crop season progressed (Chapter 8). The spatial and temporal distribution patterns of *P. xylostella* and associated parasitoids in the crops have implications for the design of accurate sampling procedures to support decisions on the need for pest control measures. In general, the assessment of *P. xylostella* infestations is made by trapping adults or counting numbers of larvae on randomly selected plants (Ulmer et al., 2004). Where populations are aggregated, traditional sampling may not provide accurate estimates of the pest and parasitoid populations, and the present study suggests that population assessment methods should be based on more rigorous research focusing on the spatial distribution of *P. xylostella*.

The strong correlation between leaf sulfur and nitrogen and spatial distributions of *P. xylostella* suggests that plant patches with high sulfur and/or nitrogen contents may serve as areas of high infestations of *P. xylostella*. The discovery of relationship between sulfur and *P. xylostella* in one study field, but not in a second field, and the discovery of relationship between nitrogen and *P. xylostella* in only one of two fields are intriguing and point to questions that should be investigated further in replicated field studies where a gradient could be established in different soil fertility levels. This could enable further exploration of this relationship. Further, in commercial field situations, there are many interacting factors that could not be measured in the present study. For example, weed infestation data were not recorded, yet it is known that weeds can interact with insect behavior (Horn, 1981; Shellhorn and Sork, 1997; Finch and Collier, 2000; Norris and Kogan, 2000; Dosdall et al., 2003; Oyediran et al., 2007). Similarly, there may have been differences in plant density between different grid plots, and these could also interact with nutrients and subsequently herbivore abundance. Nevertheless, the linkage between sulfur and nitrogen and *P. xylostella* have been carefully examined in laboratory situations (Gupta and Thorsteinson, 1960; Marazzi et al., 2004) and they appear to govern field distributions to some extent.

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